VOL. VI NOVEMBER, 1918 No. 5

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

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PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter August 23, 1917, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

Price { $6.00 per year, two volumes, United States
$6.25 per year, two volumes, Canada
$6.50 per year, two volumes, other countries

SOIL SCIENCE

Contents for November, 1918

THE EFFECT OF CERTAIN ORGANIC SUBSTANCES ON SEED GERMINATION[1]

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Received for publication, November 1, 1918

In a former publication it was reported that the addition of green manures to the soil produces unfavorable conditions for the growth of certain seedlings (3). It seems that the injurious factor, probably soil fungi, develops so rapidly that the young seedlings are killed before they appear above the surface of the soil. This is especially true in the case of seeds rich in oil. Fortunately, the majority of seeds, e.g., the cereals, corn, wheat and oats, are very resistant to the attack of the harmful fungi.

During the progress of this work with green manures data were collected concerning the influence of various organic compounds on germination. The more important results are reported at this time.

The facts presented in the earlier report show that the green manure, clover for example, favors the growth of injurious fungi. If food supply is the controlling factor in the development of the harmful fungi, then it seems probable that other forms of readily available organic matter should produce a similar effect. Accordingly experiments were made using various substances especially adapted to the growth of soil organisms.

EXPERIMENTAL WORK

The soil used in this work was the Miami silt loam taken from the Station farm. Two kilos of soil were placed in small glazed jars. All of the organic substances were added in dry form to the soil and thoroughly mixed with it. The moisture content was held at half saturation and the temperature was kept at 20°C. Each week after planting, the number of seedlings was counted and the percentage of germination recorded.

Four different substances, alfalfa, casein, peptone, and sugar, were tried in varying amounts. The following seeds were tested: alfalfa, buckwheat, castor bean, red clover, corn, cotton, flax, hemp, white lupine, mustard, oats, serradella, soybean, sunflower, sweet pea, and wheat. According to chemical analysis the seeds included in the preceding group vary greatly in composition. For example, wheat contains less than 2 per cent of fat, while

[1] Published by permission of the Director of the Wisconsin Agricultural Experiment Station.

castor beans contain more than 50 per cent of fat. The effect of composition of the seed on the injury produced will be noted from the experiments. All results reported in this paper represent the average of at least two separate tests, in many cases more. Except in the case of the small seeds, alfalfa, clover, flax, and mustard, 10 to 20 seeds were planted to each jar; for the small seeds 20 to 50.

Nitrogenous substances

The injury to germination resulting from applications of easily decomposed organic substances may be produced in two or more ways: (a) accumulation of poisonous by-products; (b) increased growth of harmful organisms. It is the aim of this paper to consider especially the biological effect of organic substances on seed germination.

In the first series of tests it was arranged to measure the effect of dry, finely powdered alfalfa, casein, and peptone on the germination of cotton seed. If the increased growth of the harmful fungi in green manured soils is due to the presence of an easily decomposed nitrogenous compound, it seems possible that alfalfa, casein, or peptone may produce a somewhat similar effect. Casein and peptone are especially suitable for the growth of micro-organisms. The effect of these substances on germinating seed is shown by the figures of table 1. Here the average percentage of germination after one, two, and three weeks is recorded.

TABLE 1

Effect of alfalfa and of casein on the germination of cotton seed

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	None	90	90	90	100
2	0.14 alfalfa	100	100	100	111
3	0.28 alfalfa	90	90	90	100
4	0.56 alfalfa	100	100	100	111
5	0.04 casein	85	85	85	95
6	0.09 casein	90	95	95	105
7	0.18 casein	100	100	100	111
8	0.35 casein	75	75		83
9	0.70 casein	60	70		77

In order to make the results comparable to those obtained from green manures when turned under, the weight and percentage of nitrogen in a representative crop were used as the basis of all calculations.

The powdered alfalfa containing 7.38 per cent of moisture was added in amounts equivalent to 0.5, 1, and 2 per cent of green tissue. The casein was added in amounts equivalent to 1, 2, 4, 8, and 16 per cent of green clover, assuming that clover contains 4.4 per cent of protein.

From the data presented in table 1 it is very clear that neither alfalfa nor casein caused any great decrease in the germination of cotton seed. It is true that in certain cases where casein was added in amounts equivalent to 8 and 16 per cent (nitrogen basis) of green clover, there was a decrease in germination. Just why the air-dry powdered alfalfa does not cause a decrease in germination similar to that produced by green alfalfa cannot be explained unless it is due to a change in chemical composition, or to a change in the flora on the alfalfa, or to both factors. Since small amounts of alfalfa or casein produced no harmful effect on germination, it was arranged to make a new test using larger amounts of the nitrogenous substances. For this purpose, powdered alfalfa, casein, and peptone were chosen. The results of the experiment are shown in table 2.

TABLE 2

Effect of nitrogenous substances on the germination of cotton seed

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	None	30.0	70.0	70.0	100
2	0.5 alfalfa	17.0	72.5	72.5	103
3	0.5 casein	7.5	62.5	62.5	89
4	1.0 casein	0	12.5	12.5	18
5	0.5 peptone	12.5	82.5	82.5	118
6	1.0 peptone	5.0	20.0	20.0	28

Here again, the data of the table failed to show any decrease in germination when dry powdered alfalfa, casein, or peptone were applied in small quantities. When added in larger quantities, more than one-half of 1 per cent, there was a decided decrease in germination. From the figures of these two tables it is plain that nitrogenous compounds such as alfalfa, casein and peptone decomposing in the soil, do not, unless they occur in large amounts, cause any change in the germination of cotton seed. The conclusion seems justified that the influence on germination of dry powdered alfalfa, casein, and peptone is very different from that of green clover or green alfalfa. In this connection the question of reaction suggests itself. Accordingly experiments were planned to see what effect casein and alfalfa powder, in amounts large enough to produce a serious injury, would have on the germination of soybeans in the presence of calcium carbonate. According to von Brehmer (1) lime increases the percentage of germination. After mixing the nitrogen compounds thoroughly with the soil, 10 soybean seeds were sown in each jar. The average percentage of germination is shown in table 3.

Unlike the results of previous tests, 0.5 per cent of casein decreased the percentage of germination, while 1 per cent of casein entirely prohibited germination. The addition of carbonate of lime with the casein failed to

TABLE 3

Effect of nitrogenous substances and calcium carbonate on the germination of soybeans

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	None	65	75	75	100
2	0.5 casein	50	50	50	66
3	1.0 casein	50	0	0	0
4	1.0 casein and 1 $CaCO_3$	20	0	0	0
5	0.5 alfalfa	45	85	85	113
6	0.5 alfalfa and 1 $CaCO_3$	45	70	75	100
7	1.0 alfalfa	45	55	55	74
8	1.0 alfalfa and 1 $CaCO_3$	50	70	70	93
9	2.0 alfalfa	30	35	35	46
10	2.0 alfalfa and 1 $CaCO_3$	40	50	50	66

overcome the harmful effect on germination. Plate 1, figure 1 is the photograph of the number of seedlings two weeks after treatment.

Where alfalfa powder alone was added to the soil, it required more than 0.5 per cent to retard germination. Although the evidence is not conclusive, it seems that limestone tends to overcome in part the injury caused by alfalfa. This difference in germination of soybeans in the treated and untreated soil is shown in plate 1, figures 2 and 3.

Nitrogen-free substances

It was found that when nitrogenous substances, i.e., alfalfa, casein, and peptone are added to the soil in quantities equivalent to the nitrogen present in an average crop of green alfalfa, normal germination occurred. There seems to be no doubt that the dry nitrogenous substances must be added in much larger amounts in order to injure germination seriously. Although the data are limited, it is reasonably safe to conclude that the presence of small amounts of these readily available nitrogenous compounds fails to bring about the proper conditions for the development of the harmful fungi. Accordingly, it was planned to measure the effect of certain carbohydrate compounds on germination. Cane sugar, or saccharose, was employed because this substance offers a very suitable carbohydrate compound for the majority of microörganisms.

In a former paper it was found that decomposing green plant tissue favors the growth of fungi which are destructive to seedlings. If the damage to the germinating seeds is due to the addition of food for the fungi, it seems probable that a soluble carbohydrate, e.g., saccharose, should produce a somewhat similar effect. It has been shown repeatedly that the addition of cane sugar to soil is followed by an enormous increase in the number of bacteria and a parallel increase in carbon dioxide evolution. The great gain in carbon

dioxide will no doubt prove detrimental to seed germination (7, 17). The references given below illustrate this point. Engberding (2) found that 2 per cent sugar (saccharose) caused an increase in the number of bacteria from about 1000 to 1500 per cent. Miller (10) obtained the following results from the use of 2 per cent of dextrose in Goettingen soil:

Number of bacteria per gram of soil

	AFTER		
	8 days	21 days	41 days
Control	9,100,000	14,800,000	17,400,000
Sugar, 2 per cent	74,800,000	189,500,000	393,200,000

The enormous gain in number is soon followed by a rapid decrease.

In order to find out the effect of sugar and of sterilization on the number of bacteria in Miami soil, a series of counts was made. The soil was placed in small jars, treated as shown below, and incubated at approximately 20°C. in the greenhouse. No precaution was taken to keep the sterilized soil free of bacteria. At regular intervals of two days each unsterilized tap water was added to keep the soil at approximately 18 per cent of moisture. In this way the sterilized soil soon became thickly seeded with bacteria. Plate counts were made two weeks after treatment in the first test and four weeks in the second test.

Two weeks after treatment

	Bacteria per gram of dry soil
1. Unsterilized soil	8,966,000
2. Sterilized soil	61,412,000
3. Sugar, 1.5 per cent	292,323,000

Four weeks after treatment

1. Unsterilized soil	58,081,000
2. Sugar, 1 per cent	203,280,000
3. Alfalfa, 0.5 per cent	358,422,000

The results are in accord with the reports of others, namely, sterilization alone usually brings about favorable conditions for the multiplication of bacteria. Sugar and alfalfa cause an enormous gain in the number of soil bacteria. Just what effect this increase in the soil flora will have on the germination of seed is shown in the figures of table 4. Here a record was kept of the average percentage germination of mustard seed and the total number of bacteria per gram of dry soil.

No precautions were taken to prevent contamination after the mustard seed were planted. It will be seen from the figures of the table that sugar was injurious to mustard seedlings (4). Moreover, the injury was corrected in part by sterilization, although sterilization alone seems to have produced a condition slightly injurious to germination. The injury to germination

from sterilization of soil has been noted from various sources (6, 14, 15). Plate 2 shows the effect of sterilization on mustard seedlings. The plants in jars 3 and 4 are much behind those of jars 1 and 2. This picture was taken fourteen days after planting.

As regards the number of bacteria it will be found that sterilization alone caused an enormous gain, which is especially noticeable after 21 days. Sugar alone caused a rapid increase in number followed by a decrease after two weeks. Just why the sterilized, sugar-treated soil at this date should contain fewer bacteria than the untreated is not known. From the data it will be seen that the harmful factor is not a result of enormous *increase* in the number of bacteria.

TABLE 4

Effect of sugar on the germination of mustard seed in sterilized and unsterilized soils

NUMBER	TREATMENT	GERMINATION			BACTERIA PER GRAM OF DRY SOIL	
		2 weeks	3 weeks	Relative	14 days	21 days
	per cent	*per cent*	*per cent*	*per cent*		
1	None	100	100	100	12,559,000	14,248,000
2	Sterilized	80	85	85	50,263,000	112,253,000
3	Cane sugar, 1	60	60	60	256,350,000	45,235,000
4	Cane sugar 1 sterilized	60	75	75	4,717,000	61,990,000

TABLE 5

Effect of varying amounts of sugar on the germination of cotton seed

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	None	42.5	80	85.0	100
2	0.25 sugar	45.0	85	85.0	100
3	0.50 sugar	50.0	85	87.5	103
4	1.00 sugar	32.5	85	87.5	103
5	2.00 sugar	27.5	80	80.0	94
6	3.00 sugar	15.0	75	77.5	91
7	5.00 sugar	0	30	0*	0

* All seedlings killed by the end of the third week.

Effect of different amounts of sugar. This experiment was prepared by adding to Miami silt loam soil cane sugar in varying amounts from 0.25 to 5 per cent. After planting the soil moisture was increased to one-half the total water-holding capacity. The results of this experiment are presented in table 5.

Here it was found that normal germination occurred in all cases with amounts of sugar from 0.25 to 1 per cent, while greater amounts proved in-

jurious. In the presence of 5 per cent all the seedlings were killed by the third week. The retarding effect of sugar on germination may be explained in part by two factors: first, the rapid evolution of carbon dioxide; second, the formation of acids.

Effect of sugar on various seeds. In order to secure additional data concerning the relation of sugar to the process of germination, seeds from the following plants were used: alfalfa, buckwheat, castor bean, corn, flax, hemp, oats, red clover, serradella, soybean, sunflower, sweet pea, and wheat. Throughout this test the same type of soil with 1 per cent of sugar was

TABLE 6

Effect of sugar on the germination of various seeds

NUMBER	SEED	TREATMENT	GERMINATION			RELATIVE
			1 week	2 weeks	4 weeks	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Alfalfa	None	82.5	82.5	82.5	100
2	Alfalfa	1 sugar	65.0	72.5	72.5	88
3	Buckwheat	None	70.0	85.0	87.5	100
4	Buckwheat	1 sugar	75.0	80.0	85.0	97
5	Corn	None	90.0	90.0	90.0	100
6	Corn	1 sugar	30.0	85.0	85.0	94
7	Flax	None	85.0	87.5	90.0	100
8	Flax	1 sugar	40.0	47.5	67.5	75
9	Hemp	None	87.5	87.5	87.5	100
10	Hemp	1 sugar	62.5	82.5	82.5	94
11	Oats	None	90.0	92.5	95.0	100
12	Oats	1 sugar	87.5	97.5	97.5	103
13	Red clover	None	55.0	62.5	62.5	100
14	Red clover	1 sugar	62.5	62.5	70.0	112
15	Serradella	None	62.5	62.5	62.5	100
16	Serradella	1 sugar	32.5	42.5	42.5	68
17	Sunflower	None	100.0	100.0	100.0	100
18	Sunflower	1 sugar	100.0	100.0	100.0	100
19	Sweet pea	None		72.5	72.5	100
20	Sweet pea	1 sugar		77.5	77.5	107
21	Wheat	None	87.5	90.0	90.0	100
22	Wheat	1 sugar	80.0	100.0	100.0	111

employed. The results of the tests are given in table 6. Because of poor germination, the results with the castor beans were omitted from the table. The figures of the table show very clearly the retarding effect of sugar on seed germination. Apparently the young seedlings soon recover from the period of depression, for within 4 weeks after treatment the percentage of germination in the presence of sugar is nearly as high as in the control. In a few cases the number of seedlings in the sugar-treated series was equally as great or exceeded that of the control. Aside from the effect on germination, the sugar-treated soils were characterized by an acid reaction, and apparently

the physical properties of the soil were changed, causing the soil to become very compact.

An illustration of the effect of sugar on seedlings is given in plate 3, figures 1 to 3, and in plate 4, figures 1 to 3.

Although no consistent decrease in percentage of germination was noted, there was a decided decrease in plant growth. The sugar-treated plants were far behind the untreated; they were not only small, but were also marked by a yellow color. Probably the pale color and abnormal development of plants in sugar-treated soils is partly a result of the lack of available nitrogen (11, 12, 16). The great increase in the number of bacteria in the treated soil is no doubt followed by a decrease in soluble nitrogen.

The results of germination tests with corn are in accord with the reports of earlier investigators. Mazé (9) and Lipman (8) noted that sugar retarded

TABLE 7

Effect of cane sugar on germination of flax, mustard, lupine, and sunflower seed

NUMBER	SEED	TREATMENT	GERMINATION		RELATIVE
			1 week	2 weeks	
		per cent	*per cent*	*per cent*	*per cent*
1	Flax	None	85.0	85	100
2	Flax	1.5 sugar	45.0	65	76
3	Mustard	None	95.0	95	100
4	Mustard	1.5 sugar	42.5	65	68
5	White lupine	None	90.0	90	100
6	White lupine	1.5 sugar	90.0	90	100
7	Sunflower	None	50.0	65	100
8	Sunflower	1.5 sugar	25.0	55	84

both the germination and the growth of corn. A somewhat similar effect was noted by Pfeiffer (13) concerning the action of sugar on oats.

Additional data in regard to the effect of sugar on germination or on very young seedlings is shown in table 7. This test represents a study of the relation of certain oil seeds to sugar.

The influence of the sugar is well marked at the end of the second week. Flax and mustard seem especially sensitive to this treatment.

Effect of sugar and limestone. If the retarding effect of sugar on germination is due to acidity, then the presence of a base should tend to overcome this condition. Although repeated tests were made using different quantities of lime, no decided effect on the germination of soybeans was noted.

Effect of sugar and lime in sterilized soil. Here three soil types, Miami silt loam, sand, and a mixture of equal parts of these two soils, were used in duplicate. It was thought that perhaps a change in the physical, as well as in the chemical properties of soil, would influence the rate of germination. For example, the products of decomposition differ in a compact soil with a

limited supply of oxygen from those in an open type of soil. Calcium oxide was used in place of calcium carbonate. The average percentage germination is given in table 8.

From the data it may be seen that in unsterilized soil sugar retarded the rate of germination. Probably the carbon dioxide formed in the decomposition of sugar was the cause. As shown from previous results, most probably the percentage of germination in the presence of sugar would have been much greater if the experiment had been continued for a week or two longer. The effect of sugar is noted by the decrease in number and size of seedlings. Unlike the seed in green manured soil, examinations after two weeks showed that many of the seeds were just beginning to sprout. The sugar *retarded* germination but did not cause the seed to *decay*.

TABLE 8

Effect of sugar and lime on the germination of cotton seed

NUMBER	TREATMENT	GERMINATION AFTER 2 WEEKS		
		Clay soil	Sandy soil	Sandy clay soil
	per cent	*per cent*	*per cent*	*per cent*
1	None	90	30	90
2	Sterilized	100	90	80
3	0.2 CaO	90	70	80
4	0.2 CaO, sterilized	90	90	90
5	1.5 sugar	60	30	60
6	1.5 sugar, sterilized	90	90	90
7	1.5 sugar 0.2 CaO	60	50	70
8	1.5 sugar, sterilized 0.2 CaO	95	90	90

SUMMARY

1. Nitrogenous substances such as alfalfa powder, casein, and peptone do not seriously injure seed germination unless used in very large quantities.

2. As compared with green manure (nitrogen content), very large amounts of casein and peptone are required to cause a noticeable decrease in germination.

3. Calcium carbonate apparently does not lessen the decrease in germination due to very large applications of alfalfa powder or casein.

4. Sugar greatly increases bacterial growth and retards the rate of seed germination. In large amounts it decreases the percentage of germination.

5. The retarding action of sugar on the germination of seeds is perhaps due to the large amount of carbon dioxide given off in the decomposition of the sugar.

6. Soil sterilization often inhibits the rate of seed germination.

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PLATE 1

EFFECT OF CASEIN AND ALFALFA ON GERMINATION OF SOYBEANS

Fig. 1. Jars 1 and 2 untreated; 3 and 4 received 0.5 per cent casein; 5 and 6 received 1.0 per cent casein, and 7 and 8 received 1.0 per cent casein and 1.0 per cent limestone.

Fig. 2. Jars 1 and 2 untreated; 3 and 4 received 0.5 per cent dry alfalfa hay; 5 and 6 received 1.0 per cent dry alfalfa hay, and 7 and 8 received 2.0 per cent dry alfalfa hay.

Fig. 3. The jars in this series received the same treatment as those in figure 2 plus 1.0 per cent limestone.

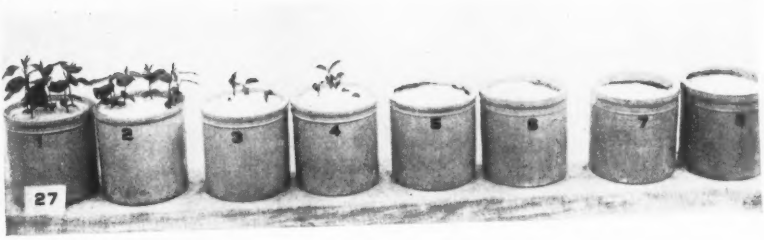

FIG. 1

FIG. 2

FIG. 3

PLATE 2

EFFECT OF STERILIZING SOIL ON MUSTARD GERMINATION

The soil in jars 1 and 2 was untreated; in jars 3 and 4 it was sterilized for 3 hours in an autoclave.


1
2
3
4
19

PLATE 3

EFFECT OF SUGAR ON GERMINATION

Jars 1 and 2 untreated, 3 and 4 received 1 per cent cane sugar.
Fig. 1. Corn
Fig. 2. Oats
Fig. 3. Wheat

FIG. 1

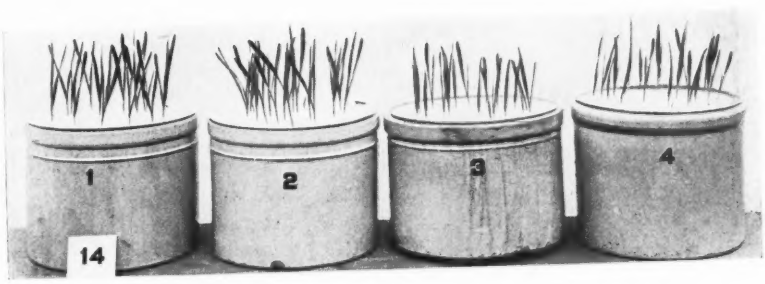

FIG. 2

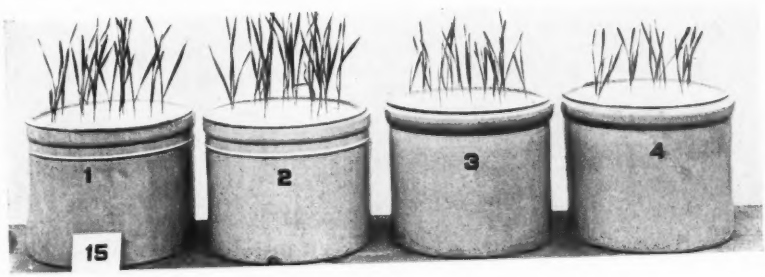

FIG. 3

PLATE 4

EFFECT OF SUGAR ON GERMINATION

Jars 1 and 2 untreated; 3 and 4 received 1 per cent cane sugar.
Fig. 1. Buckwheat
Fig. 2. Hemp
Fig. 3. Flax

FIG. 1

FIG. 2

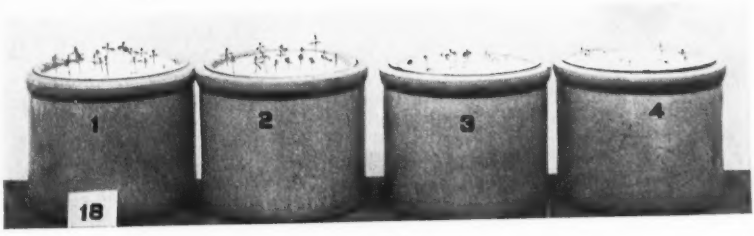

FIG. 3

EFFECT OF SULFOFICATION AND NITRIFICATION ON ROCK PHOSPHATE[1]

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Received for publication November 12, 1918

Recent investigations have directed attention to certain biochemical processes as effective agents for converting the phosphorus of rock phosphate into a more available form. The action of the acidity produced by oxidation of sulfur has been proposed by Lipman and associates (4) as a means for rendering the phosphorus of rock phosphate available when mixed with soil and sulfur in compost heaps. The solvent effect of nitrous acid formed during the course of nitrogen transformations in soil is considered by Hopkins and Whiting (2) to have an important bearing on the question of availability of insoluble phosphates. They state that 115 pounds of phosphorus soluble in water were secured by the oxidation of 56 pounds of nitrogen. The conclusions of the latter investigators are based on experimental data obtained from mixtures of purified calcium phosphate and ammonium sulfate in solution cultures.

On account of the increasing cost of acid phosphate during the past two years, studies of the effects of sulfur oxidation and nitrification on phosphates were undertaken at the Ohio Station in November 1917. That phase of the investigation pertaining to the effect of nitrification on availability of phosphates naturally present in soil or added to it, was taken up in connection with the subject of sulfur oxidation. The investigation was so arranged that the influence of nitrification on phosphates could be determined on the same soil mixtures that were used for studying the effect of sulfur oxidation on phosphates.

The question of whether the method of composting as proposed by Lipman and associates would be practical for Ohio conditions, influenced us to plan our experiments so that the quantity of rock phosphate used would more nearly approach that which is applied in field practice, notwithstanding that the opportunity for action of the acidity on the phosphate under these conditions would be much less than if larger amounts of the materials were intimately mixed together. Rock phosphate and sulfur were therefore used in a preliminary experiment at the rate of 1000 pounds per million of soil. While the results obtained showed that the sulfur was as readily oxidized as if a larger amount had been used, and no difficulty was experienced in measuring

[1] G. E. Boltz and J. A. Stenius have assisted with the analytical work.

the amount oxidized, it was found impossible to determine to what extent the availability of the phosphorus had been changed on account of the adsorption capacity of the soil for phosphorus. It was therefore deemed advisable to use larger amounts than would be practical in the field, in order to obtain indications of interactions which had occurred under different conditions of treatment, and this was done in the experimental work begun in November, 1917.

More recently Kelley (3) has reported the results of an investigation which included a study of the effect of the oxidation of soil nitrogen, and that supplied by dried blood and ammonium sulfate in soil mixtures with and without the addition of calcium carbonate. His procedure for the study of the solubility of phosphate more nearly approximates field conditions than do those experiments where the conclusions were based on results obtained from solution cultures.

The general plan of the experimental work reported in this paper is somewhat similar to that followed by Kelley in his study of the influence of nitrification on solubility of phosphates, in that dried blood and ammonium sulfate were used as sources of nitrogen, and additions of calcium carbonate were made in some instances to determine whether the acidity developed by the processes would have a selective action on the calcium of added phosphate in soils naturally calcareous.

EXPERIMENTAL PROCEDURE

A series of 56 mixtures which included various treatments of soil, peat and a pure quartz sand were used as mediums for studying the action of nitrification and sulfofication. An additional series of sand cultures were used for a further study of the effect of nitrification on phosphorus, for the reason that in some preliminary work with soils indications had been furnished by the calcium in water solutions of soil treated with rock phosphate and organic nitrogen, that the phosphate had been acted on by some agency, although no increased amount of soluble phosphorus was found. This was considered to be due to the absorbtive capacity of the soil for phosphorus.

The soils, which were a silt loam and a black clay, differed considerably in their composition and physical characteristics. The acid silt which was used for the greater number of the experimental mixtures has a requirement for base equivalent to 4000 parts of calcium carbonate per million of soil, and is rather deficient in organic residues; it has a total nitrogen content of 1000 parts per million. The black clay used is naturally basic and contains 4000 parts of nitrogen per million. The phosphorus content of the black clay is about 1000 parts per million and the sulfur 500. These elements are present in smaller amounts in the silt loam, the phosphorus content being 450, and the sulfur 200 parts per million of soil. The soil was air dried and ground finer than 2 mm.

Two forms of nitrogen were used, dried blood and ammonium sulfate In the mixture when either of these carriers were included as a part of the treatment, 4 gm. of blood and 4 gm. ammonium sulfate were added to 500 gm. portions of soil.

A Tennessee brown rock phosphate analyzing 12 per cent phosphorus furnished the supply of insoluble phosphorus in certain mixtures. When sulfur was included as a part of the treatment the amount added was 2 gm.

Additions of varying amounts of calcium carbonate were made to certain mixtures of the acid silt loam soil in order to study the effect of the processes in an acid soil and in soil supplied with basic material. The amounts of ca'cium carbonate added to certain mixtures of the silt loam are expressed in the tabulations of results. The silt loam soil has a requirement for calcium carbonate which is slightly less than 4000 pounds per million of soil. The largest addition of calcium carbonate was at the rate of 8000 pounds per-million, and decreasing amounts provided for different degrees of basicity.

After the various treatments were thoroughly mixed with the 500 gm. portions of soil, sufficient water was added to satisfy 60 per cent of the water holding capacity, and the mixtures transferred to the quart jars used for containers. Duplicates of all mixtures were prepared. The contents of the uncovered jars were stirred after each addition of water, which was made every fourth day, so that the supply of oxygen necessary for the activities of the organisms was provided. The soil mixtures were incubated at a temperature of 30°C. for a period of 19 weeks from November 21, 1917 to April 15, 1918.

Previous to adding water, portions of the mixtures were withdrawn and citrate soluble phosphorus determined to measure any change in the solubility of phosphorus in rock phosphate and soil, resulting from possible interactions of the materials before biological activities had progressed.

The indications of the effect of sulfur oxidation and nitrogen transformations on availability of phosphorus at the end of the experimental period, April 10, 1918, were obtained from the phosphorus soluble in neutral ammonium citrate solution. This determination was made according to the regular method for available phosphorus in fertilizing materials, the soil mixtures being air dried and thoroughly mixed previous to extracting with the citrate solution.

Measurements of nitrification and the extent to which oxidation of sulfur had proceeded were secured by extracting 400 gm. portions of the mixt res with 2500 cc. of distilled water for 15 hours, with continuous shaking for 3 hours.

Portions of the water extract filtered through Berkfield filters were used for determinations of nitrates, water soluble sulfur and sulfates, and calcium.

Nitric nitrogen was determined by the modified Devarda reduction method (1). The water extracts of the soil mixtures were tested for nitrites, but no appreciable quantities were present, the largest amount found in any

of the mixtures being less than 1 part per million. The relative acidity of the water extracts was determined by titration, using methyl red for the indicator. Calcium was determined volumetrically, and sulfur precipitated as barium sulfate, according to the approved procedures.

EFFECT OF SULFUR OXIDATION

A considerable quantity of sulfur in a finely divided condition, mixed with soil at the rate of 4,000 parts per million of soil has been converted into sulfuric acid as indicated by the sulfates in the water solution at the expiration of the incubation period. Approximately 50 per cent of the added sulfur has been oxidized, the amount recovered varying somewhat, depending upon the soil and the materials included as part of the treatment.

Aside from a plentiful supply of oxygen, there are other factors which appear to be necessary for the active development of the sulfur-oxidizing

TABLE 1

Effect of sulfur oxidation on rock phosphate in sand after 19 weeks incubation (Data expressed as parts per million of dry sand mixtures)

ADDITIONS TO 500 GM. SAND	CITRATE-SOLUBLE PHOSPHORUS	WATER-SOLUBLE	
		Calcium	Sulfur
	p. p. m.	*p. p. m.*	*p. p. m.*
None	7	7	30
Sulfur, 2 gm	8	33	42
Sulfur, 2 gm.; calcium carbonate 2 gm	8	656	503
Rock phosphate, 8 gm	214	46	31
Rock phosphate 8 gm.; sulfur, 2 gm	242	150	166

organisms. A series of mixtures where sand was used as a medium show the effect of the presence of a basic calcium compound in promoting sulfur oxidation. From the results tabulated in table 1, it will be observed that in a sand medium the presence of calcium carbonate has promoted the oxidation of sulfur, since in the sand mixtures where it was present, the quantity of sulfur oxidized increased from 42 to 503 parts per million which indicates that some neutralizing agent for the acid formed, as calcium carbonate in this case, is essential for the continued activities of the sulfofying organisms. The results in table 2, for sand mixtures containing a smaller amount of sulfur, also show the influence of calcium carbonate. In both these series of mixtures no provision was made for inoculation, but regardless of this, the oxidation of sulfur was quite active when calcium carbonate was included.

Conflicting indications are obtained with respect to the effect of calcium carbonate on sulfofication in sand, and in soil cultures, since the results for soil mixtures which will be discussed later, show that the presence of calcium carbonate has tended to depress sulfofication.

If the results for the sand cultures are considered to furnish the more correct explanation concerning the influence of calcium carbonate on sulfofication, the effect its addition has had can be interpreted as meaning that a basic compound is necessary. The results for sulfur oxidized in the acid soil show that bases other than calcium serve to neutralize the acidity produced, because this soil contains no calcium carbonate, and only a small amount of calcium in other combinations.

The smaller increase in oxidized sulfur which occurred in sand cultures where rock phosphate was used with sulfur in the absence of calcium carbonate, indicates that as a source of base necessary for the activities of the sulfofying organisms, rock phosphate is much less effective than calcium carbonate. This is also apparent from the results for nitrification of blood and ammonium sulfate in acid soil mixtures which included rock phosphate with these nitrogen carriers.

The effect of calcium carbonate on sulfur oxidation in an acid soil is shown in table 3, which gives the data pertaining to the effect of sulfur oxidation on

TABLE 2

Sulfur oxidation and availability of phosphorus in sand mixtures, after 17 weeks incubation.
(Data expressed as parts per million of dry sand mixtures)

ADDITIONS TO 500 GM. SAND	CITRATE-SOLUBLE PHOSPHORUS	WATER-SOLUBLE	
		Calcium	Sulfur
	p. p. m.	*p. p. m.*	*p. p. m.*
Rock phosphate, 0.5 gm	30	25	0
Rock phosphate 0.5 gm., sulfur, 0.5 gm	60	98	70
Rock phosphate 0.5 gm., sulfur 0.5 gm., calcium carbonate 2 gm	25	722	591

rock phosphate in soil mixtures. It may be that phosphorus, although converted into a more soluble form, will be fixed by soil to such an extent that a correct measure of changes in its availability, due to the action of acidity resulting from the oxidation of sulfur or other biochemical process, can not be obtained from the phosphorus results alone.

If there is any appreciable reaction between insoluble calcium phosphate and sulfur acidity, with the formation of calcium sulfate, the calcium in the water solution may furnish a more reliable indication than will be given by the figures for phosphorus soluble in neutral ammonium citrate solution. Where rock phosphate and sulfur were in contact in sand mixtures the fixation of phosphorus was no doubt largely, if not altogether, eliminated, and the citrate-soluble phosphorus as well as the calcium should furnish evidence of any appreciable action the sulfur acidity has had on the rock phosphate.

The phosphorus results for the sand mixtures given in table 1 show some increases in the availability of phosphorus at the end of the 19 week period, where rock phosphate and sulfur were used together. A similar result was

also obtained in the group of mixtures where sulfur and rock phosphate were added at the rate of 0.5 gm. to 500 gm. of sand, table 2.

When an acid soil was employed as a medium, the results obtained are of more interest on account of the wider range of treatment. In this soil series, the data for which are given in table 3, the addition of calcium carbonate provided for different degrees of basicity.

It will be noted that the effect of calcium carbonate when added in amount sufficient to satisfy the soil's requirement has been to depress the oxidation of sulfur as indicated by SO_4 in the water extract. When approximately half of the soils requirement was satisfied the quantity of sulfur oxidized increased,

TABLE 3

Effect of sulfur oxidation in acid silt loam soil, after 19 weeks incubation. (Data expressed as parts per million of dry soil mixtures)

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acidity*	Alkalinity*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
None	45	60	111	37	84	0	3
Calcium carbonate, 2 gm.	48	54	270	50	128	0	14
Sulfur, 2 gm.	44	99	370	2,143	6	338	0
Sulfur, 2 gm.; calcium carbonate, 4 gm.	53	72	858	1,294	35	4	0
Sulfur, 2 gm.; calcium carbonate, 2 gm.	45	74	785	1,923	21	194	0
Rock phosphate, 8 gm.	53	98	114	42	105	0	2
Rock phosphate, 8 gm.; Calcium carbonate, 2 gm.	67	96	280	64	128	0	14
Rock phosphate, 8 gm.; sulfur, 2 gm.	50	630	728	1,900	6	192	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; Calcium carbonate, 4 gm.	59	120	1,361	1,401	50	6	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; Calcium carbonate, 2 gm.	59	132	981	1,592	12	75	0

*Acidity expressed as H_2SO_4, and alkalinity as $CaCO_3$

but was less than the amount obtained in solution from the soil mixture to which sulfur only was added.

The quantities of citrate-soluble phosphorus found at the beginning of the experiment in the soil mixtures receiving additions of rock phosphate and sulfur do not differ materially from those in the soil receiving no phosphorus, as would be expected, since no incubation period had intervened between the time the materials were incorporated and the time when samples were withdrawn for measuring any changes which had occurred. At the end of the 19 week period there was a small increase in all of the mixtures, the most pronounced increases, aside from those observed where rock phosphate and sulfur were in contact, were found in the soils receiving additions of rock phosphate and sulfur separately.

The only considerable increase noted was that resulting from oxidation of sulfur incorporated with rock phosphate where no other additions were made. The proportion of rock phosphate to soil was such that phosphorus was added at the rate of 1900 parts per million. The effect of sulfur when incorporated with rock phosphate, in the absence of calcium carbonate or nitrogen carriers, was responsible for the accumulation of 630 parts per million of available phosphorus on the basis of the dry soil.

While the presence of calcium carbonate has depressed the oxidation of sulfur, the quantity of sulfates found indicates that there was sufficient acidity to have attacked the rock phosphate, providing that no calcium carbonate had been present. The results for available phosphorus in the acid soil mixtures, to which additions of calcium carbonate were made, show that the action of oxidized sulfur on rock phosphate will be decreased considerably in soils containing calcium carbonate. Further evidence of this is furnished by the results for the black clay soil which is decidedly basic although its natural calcium carbonate content is only 300 parts per million.

The basic clay used as a medium contains phosphorus in a form readily acted on by the acidity produced by sulfofication, according to the indications given by the results where no rock phosphate was added either alone or in combination with a nitrogen carrier. It will be observed from the results for the basic clay soil, which are given in table 4, that while the oxidation of sulfur has proceeded actively, the quantity of sulfates extracted with water being similar to that from the acid soil, rock phosphate has not been attacked to the same extent. Where rock phosphate and sulfur together were the additions, the citrate soluble phosphorus increased from 129 parts per million at the beginning of the experiment to 479. In all instances where sulfur was in contact with rock phosphate, its oxidation product attacked the phosphate, but the amount of citrate soluble phosphorus was in no case as large as that obtained when sulfur was used alone with rock phosphate in the acid soil. The natural basicity of the black clay furnishes the explanation of this difference. The concentration of calcium in the water extract of the basic soil shows that it is well supplied with soluble calcium. From the fact that when a nitrogen carrier was included as a part of the treatment with sulfur, its nitrification was depressed considerably as compared with that taking place when either dried blood or ammonium sulfate was included in the soil mixture without sulfur, it is shown that the natural basicity was only slightly in excess of the sulfur acidity requirement. The calcium in solution where rock phosphate and sulfur were included together is nearly equivalent to the increased phosphorus soluble in neutral ammonium citrate.

The additions of phosphorus and sulfur made to the peat soils were less than for the other soil series, 0.5 gm. of these materials being supplied. This soil is considered to be acid, and the considerably higher nitrogen figures when calcium carbonate was added support this opinion. The results for peat soil mixtures are given in table 5. It will be noted that the oxidation of

TABLE 4

Effects of sulfur oxidation and nitrification in basic black clay, after 19 weeks incubation. (Data expressed as parts per million of dry soil mixtures)

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acidity*	Alkalinity*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
None	130	141	245	87	153	4	0
Sulfur, 2 gm.	119	270	1,173	2,156	16	42	0
Dried blood, 4 gm.	130	134	668	90	491	0	3
Dried blood, 4 gm.; sulfur, 2 gm.	129	244	1,763	2,225	12	17	0
Ammonium sulfate, 4 gm.	125	156	1,297	1,696	280	0	7
Ammonium sulfate, 4 gm.; sulfur, 2 gm.	124	259	2,080	3,480	42	30	0
Rock phosphate, 8 gm.	140	160	249	71	153	0	8
Rock phosphate, 8 gm.; sulfur, 2 gm.	129	479	1,746	2,184	0	52	0
Rock phosphate, 8 gm.; dried blood, 4 gm.	142	145	702	114	516	0	3
Rock phosphate, 8 gm.; dried blood, 4 gm.; sulfur, 2 gm.	145	323	1,849	2,398	12	23	0
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.	153	180	1,668	2,267	326	0	2
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.; sulfur, 2 gm.	139	240	2,395	3,649	40	27	0

*Acidity expressed as H_2SO_4 and alkalinity as $CaCO_3$.

TABLE 5

Sulfur oxidation and availability of phosphorus in acid peat soil mixtures, after 19 weeks incubation. (Data expressed as parts per million of dry mixtures.)

ADDITIONS TO 500 GM. PEAT	CITRATE SOLUBLE PHOSPHORUS	WATER-SOLUBLE		
		Calcium	Sulfur	Nitric nitrogen
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*
None	356	264	118	255
Calcium carbonate, 5 gm.	358	737	108	556
Sulfur, 0.5 gm.	365	657	937	105
Sulfur, 0.5 gm.; calcium carbonate, 5 gm.	365	697	818	112
Sulfur, 0.5 gm.; calcium carbonate, 2.5 gm.	335	697	942	80
Sulfur, 0.5 gm.; calcium carbonate, 1 gm.	335	641	981	58
Rock phosphate, 0.5 gm.	339	258	108	210
Rock phosphate, 0.5 gm.; calcium carbonate 5 gm.	365	737	87	560
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.	368	673	952	100
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 5 gm.	339	818	1,025	108
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 2.5 gm.	366	705	935	98
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 1 gm.	389	601	881	105

sulfur in the peat soil to which no calcium carbonate was added has increased the soluble calcium concentration. This is interpreted as resulting from the action of sulfur acidity on calcium, present in other forms than basic calcium compounds. The oxidation of sulfur in contact with rock phosphate in these peat mixtures has not increased the soluble calcium over that taken into solution from the peat mixtures which included no other treatment than sulfur. Neither do the results for phosphorus furnish indication of any action of oxidized sulfur on rock phosphate in the peat soil.

EFFECT OF OXIDATION OF SULFUR USED WITH NITROGEN CARRIERS

A series of soil mixtures, in which either dried blood or ammonium sulfate was included with sulfur and rock phosphate as a part of the treatment, were incubated for the purpose of studying the combined action of sulfofication and nitrification on rock phosphate in the presence of calcium carbonate as well as in the soil which contains no carbonate and possesses the characteristics of soils which are regarded as acidic on account of their deficiency in bases. The basic soil, without addition of calcium carbonate, was also used as a medium for the same treatments without the addition of calcium carbonate.

The results showing effect of the oxidation of sulfur, when used with nitrogen carriers, on rock phosphate in an acid soil are presented in table 6. The effect of similar treatment in the basic clay is shown by the data included in table 4.

When sulfur and blood were in contact in the acid soil, the oxidation of sulfur proceeded actively. Nitrification, however, in the absence of calcium carbonate, was practically inhibited by the acidity resulting from oxidation of sulfur. The transition from proteid to other forms of nitrogen proceeded to a slight extent only beyond ammonia, as the conditions were not favorable for the further change to nitric nitrogen.

The ammonia formed has apparently partially neutralized the acidity produced by the activities of the sulfofying organisms, since the results obtained for phosphorus, when dried blood was added to rock phosphate and sulfur in the soil mixtures, show that considerably less phosphorus was changed into a form soluble in neutral ammonium citrate, than where sulfur and rock phosphate were in contact without the addition of dried blood.

Where ammonium sulfate was included with rock phosphate and sulfur there was a further decrease in the amount of citrate soluble phosphorus. This effect on the solubility of rock phosphate when ammonium sulfate was used could not be due to a neutralization of acidity.

In the basic clay the effect of dried blood when used with sulfur has not been as marked as in the acid soil.

TABLE 6

Results showing influence of the oxidation of sulfur during 19 weeks incubation, when used in combination with nitrogen carriers, on rock phosphate in acid silt loam soil. (Data expressed as parts per million of dry soil mixtures)

ADDITIONS TO 500 GM. SOIL	CITRATE-SOLUBLE PHOSPHATE		WATER-SOLUBLE				ACIDITY*	ALKALINITY*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitrate nitrogen	Ammoniacal nitrogen		
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Rock phosphate, 8 gm.; sulfur, 2 gm.	50	630	728	1,900	6	65	192	0
Rock phosphate, 8 gm.; dried blood, 4 gm.	90	91	366	64	338	27	0	2
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.	80	279	798	2,666	6	361	254	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 4 gm.	62	62	1,295	1,628	161	225	27	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 2 gm.	57	57	917	1,977	18	313	69	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 1 gm.	59	323	694	1,926	8	340	112	0
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.	58	110	429	1,904	56	1,211		
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.	69	179	645	2,666	8	1,486	0	3
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.; calcium carbonate, 8 gm.	55	94	4,107	2,596	823	36	13	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.; calcium carbonate, 4 gm.	64	61	2,855	2,711	365	468	0	4

*Acidity expressed as H_2SO_4, and alkalinity as $CaCO_3$.

Nitrification and availability of phosphorus

The results for nitrification as affecting the availability of phosphorus which are given in table 7 show that the process proceeded more actively when the basicity of the soil was increased. Addition of calcium carbonate to this soil has stimulated the nitrification of its natural nitrogen supply, as well as that furnished by additions of dried blood and ammonium sulfate.

When calcium carbonate was not present, dried blood was nitrified to a greater extent than ammonium sulfate, the accumulation of nitric nitrogen from ammonium sulfate being less than was present in the untreated acid soil. The amount of nitrate nitrogen in the untreated soil was 84, and that produced from dried blood 294 parts per million.

The increased base requirement for the formation of nitrates from ammonium sulfate, as compared with dried blood, is clearly shown by the amount of nitric nitrogen produced from these two forms of nitrogen when the same amounts of calcium carbonate are used, that for dried blood being 462 parts per million, in contrast to only 100 parts from ammonium sulfate. Calcium carbonate was added to these mixtures at the rate of 4000 parts per million.

In soil mixtures receiving an addition of calcium carbonate at the rate of 8000 parts per million (which is double the lime requirement of the soil),

TABLE 7

Effect of nitrification in acid silt loam soil, after 19 weeks incubation. (Data expressed as parts per million of dry soil mixtures)

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acidity*	Alkalinity*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*	*p. p. m.*
None	45	60	111	37	84	0	3
Calcium carbonate, 2 gm.	48	54	270	50	128	0	14
Dried blood, 4 gm.	53	63	255	58	294	0	2
Dried blood, 4 gm.; calcium carbonate, 2 gm.	51	54	736	95	462	0	14
Ammonium sulfate, 4 gm.	42	81	355	1,852	54	0	0
Ammonium sulfate, 4 gm.; calcium carbonate, 4 gm.	42	95	2,198	1,689	569	0	3
Ammonium sulfate, 4 gm.; calcium carbonate, 2 gm.	45	49	1,070	2,070	100	0	6
Rock phosphate, 8 gm.	53	98	114	42	105	0	2
Rock phosphate, 8 gm.; calcium carbonate, 2 gm.	67	96	280	64	128	0	14
Rock phosphate, 8 gm.; dried blood, 4 gm.	90	91	366	64	338	0	1
Rock phosphate, 8 gm.; dried blood, 4 gm.; calcium carbonate, 2 gm.	63	97	667	93	394	0	13
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.	58	110	429	1,904	54	0	3
Rock phosphate, ammonium sulfate, 4 gm.; calcium carbonate 4 gm.	59	95	2,058	1,919	424	0	4

*Acidity expressed as H_2SO_4, and alkalinity as $CaCO_3$.

independent of that required for neutralizing acidity developed by the oxidation of either nitrogen or sulfur, the formation of nitrate nitrogen from ammonium sulfate exceeded the amount produced from dried blood.

Rock phosphate in the absence of calcium carbonate has slightly favored the nitrification of dried blood but not that of ammonium sulfate in the case of this acid soil. The amount of nitric nitrogen in the ammonium sulfate mixture is in this case the same as that produced from ammonium sulfate without rock phosphate.

Although the nitrification of dried blood was stimulated somewhat by the presence of rock phosphate and an increased amount of calcium was found in the water solution, the figures for phosphorus do not give any indication of the solvent action of nitrous acid on tricalcium phosphate. Granting that any change with respect to the phosphorus may have been masked by the adsorption capacity of the soil, and considering that the increased calcium more correctly reflects any interaction which occurred, the quantity of phosphorus as dicalcium phosphate equivalent to the increased calcium is only 86 parts per million of soil. Ammonium sulfate, from the indications furnished by the calcium as well as phosphorus, has slightly affected the solubility of tricalcium phosphate, but whatever action ammonium sulfate had is attributed to the sulfate ion rather than to biochemical action, since active nitrification of ammonia did not occur in the acid soil unless calcium carbonate was added.

The fact that more calcium from calcium carbonate was taken into solution from soil treated with ammonia sulfate than by the sulfur acidity following oxidation of sulfur, can be explained by the nitric nitrogen figures when the largest addition of calcium carbonate was necessary to furnish sufficient base for active nitrification; and by the sulfate ion where one half this quantity of calcium carbonate was not adequate for the formation of any considerable amount of nitric nitrogen from ammonium sulfate.

In the absence of rock phosphate or calcium carbonate, the nitrification of dried blood, as well as the action of ammonium sulfate without oxidation of its nitrogen, has increased the soluble calcium content. This is evidence that the natural calcium of this soil, existing chiefly as silicates, and partly in other combinations is almost, if not altogether, as readily attacked as rock phosphate. In considering the functioning of rock phosphate as a base and as promoting nitrification, it should be stated that the rock phosphate used contained a small amount of calcium carbonate, approximately 3 per cent.

The data for the basic soil mixtures which are given in table 4, show that there was active nitrification of blood, which was accompanied by a greater concentration of water soluble calcium than was found for the untreated soil. Although the natural basicity of the clay soil supported a more limited nitrification of ammonium sulfate, the amount of calcium converted into calcium sulfate through its action was almost double the calcium changed into a water soluble form through the instrumentality of the nitrifying process where dried blood was present.

Including rock phosphate as a part of the treatment has slightly increased the production of nitric nitrogen from both dried blood and ammonium sulfate, and it is assumed that the slightly larger amounts of calcium, which accompanied the increased accumulation of nitrates in the basic soil, were obtained from the rock phosphate.

In the group of mixtures where rock phosphate was added to a peat soil, no additions of dried blood or ammonium sulfate were made. The quanti-

ties of nitric nitrogen produced in the peat where rock phosphate was added, furnish no indication that it has supplied basicity necessary for the nitrifying process. Where calcium carbonate was added without sulfur, there was an increased accumulation of nitrates. The solubility of rock phosphate incorporated with the peat, does not appear to have been changed.

The results for citrate soluble phosphorus, water soluble calcium, and nitric nitrogen, do not indicate that rock phosphate incorporated with soil has been freely acted upon by the products formed during the transformation of either organic nitrogen or ammonium sulfate to nitrates. By contrasting the phosphorus availability where nitrogen carriers were included in the soil mixtures with those where sulfur was in contact with rock phosphate, it will at once be apparent how feeble the action of nitrification has been.

So far as the data which has been obtained under the experimental conditions described furnishes any information, the nitrification of dried blood and ammonium sulfate as an agency for rendering the tricalcium phosphate of floats available in amount sufficient for the requirement of plants must be regarded as a contributing factor rather than as a factor adequate in itself.

More significance should probably be attached to nitrification as an indirect agency, in conjunction with rock phosphate, for increasing crop yields. With a supply of available nitrogen furnished, plant growth is stimulated so that the phosphorus of rock phosphate can be utilized to better advantage.

Field results at the Ohio Station (5) confirm this opinion. Where nitrate of soda was added to soil which had received heavy applications of rock phosphate, the yield of wheat was much larger than that produced by rock phosphate without a supply of available nitrogen.

SUMMARY

The effects of sulfofication and nitrification on the availability of rock phosphate have been studied by incorporating sulfur, dried blood and ammonium sulfate with rock phosphate in soil.

In considering the influence biochemical processes may have on the availability of inert phosphates, the fact that larger additions were made than would be practical for soil application does not preclude the possibility of similar reactions occurring, although less actively, in field soils.

In an acid soil the oxidation of sulfur proceeded vigorously, approximately 50 per cent being changed to form of sulfate.

While sulfofication was somewhat depressed in an acid soil by the addition of calcium carbonate, in sand mixtures the presence of calcium carbonate was essential.

In the absence of other bases the calcium of rock phosphate did not serve as a base for the sulfofying process to any appreciable extent.

The proportion of rock phosphate to soil was such that phosphorus was added at the rate of 1900 parts per million. The oxidation of sulfur incor-

porated with rock phosphate in the absence of calcium carbonate or nitrogen carriers, has changed 630 parts of phosphorus into a form soluble in neutral ammonium citrate solution.

When calcium carbonate was added to the mixture prepared with an acid soil, the oxidation of sulfur had practically no effect on rock phosphate.

In a basic soil, the acidity resulting from sulfofication was partially neutralized by calcium naturally present as carbonate and in other combinations, so that the solvent action on rock phosphate was much less than occurred in the acid soil.

Ammonium sulfate affected the solubility of rock phosphate very little. Whatever action ammonium sulfate has had, is attributed to the sulfate ion, rather than to biochemical action, since nitrification of ammonia did not occur in a soil deficient in bases unless calcium carbonate was added.

Active nitrification of dried blood and ammonium sulfate occurred in the mixtures when conditions were favorable.

Nitrification has been stimulated by rock phosphate to a very limited extent. This fact, independent of the results for either phosphorus or calcium solubility, is sufficient indication that the process has had no appreciable action on rock phosphate in soil.

Nitrification of dried blood, so far as the citrate soluble figures furnish evidence of availability, is not an active agent for increasing the solubility of rock phosphate mixed with soil.

In the absence of rock phosphate or calcium carbonate, the nitrification of dried blood as well as the action of ammonium sulfate, independent of the oxidation of its nitrogen, has increased the concentration of water soluble calcium. More calcium has been taken into solution from the soil than from added rock phosphate. This is evidence that the calcium of the soil, existing chiefly as silicates and partly in other combinations is almost, if not altogether, as readily attacked as rock phosphate.

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ORGANIC PHOSPHORUS OF SOIL: EXPERIMENTAL WORK ON METHODS FOR EXTRACTION AND DETERMINATION

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Received for publication October 18, 1918

INTRODUCTORY

In pursuance of investigations pertaining to the availability and combinations of the phosphorus in Ohio soils, begun in 1916, the need for a method, by means of which the inorganic and the organic phosphorus of soil extracts may be differentiated, became apparent. A limited amount of experimental work had already been devoted to modification of the methods of Forbes and associates (4) for inorganic phosphorus in plant substances, with some encouraging results, when Potter and Benton's (15) adaptation of the Forbes and Emmet and Grindley (3) method for inorganic phosphorus determinations appeared.

The procedure of Potter and Benton has been made the subject of a critical study, having in view the determination of the conditions necessary for best results and the simplification of the method, if possible.

Some attention has also been devoted to the determination of total phosphorus in alkali extracts of soil; obviously, in any study of the organic phosphorus present in such extracts, where the latter figure must be obtained by difference, the accuracy of each determination is equally important.

An effort has been made to establish the conditions under which the maximum amount of organic phosphorus can be extracted from the soil, and to prove that the extracted phosphorus as determined to be organic is really in that state of combination and not inorganic phosphorus absorbed or held in an insoluble state by suspended matter, organic or inorganic.

The total organic matter (humus) content, comparative intensity of color and principal ash constituents have been determined on a number of the ammonia extracts obtained during the course of this study in the hope that a clue to their relationship to the phosphorus content might be obtained.

EXPERIMENTAL

Soil

The soil employed for this investigation was from a large lot taken for vegetation tests from the Paulding County (Ohio) experiment farm. It was formerly classed as a Clyde clay, now called "Fulton clay" by the Ohio Soil

Survey. The following quotation is from Bulletin 323 of the Ohio Station, to which the interested reader is referred for information relative to crop yields and fertility tests on this soil:

> This soil is a dark-colored, fine-textured material, water leveled over a tough glacial till, which usually forms the lower subsoil and is therefore nearly level. It has the greatest amount of available plant-food of all soils in the state.

A partial analysis of a sample of this soil, taken in close proximity to the place where the larger sample used in this investigation was obtained, is presented in table 1.

As may be seen by a comparison of the figures for ammonia-soluble organic matter and phosphorus with those presented in later tables, the sample analyzed differs somewhat in composition from the lot used in the work to be

TABLE 1

Composition of soil fom Paulding County Experiment Farm

DEPTH	PHOSPHORUS			POTASSIUM		CALCIUM		MAGNESIUM		$CaCO_3$	NITROGEN	NH_4OH – SOLUBLE HUMUS
	Total	N/5 HNO_3 – soluble	NH_4OH – soluble organic	Total	N/5 HNO_3 – soluble	Total	N/5 HNO_3 – soluble	Total	N/5 HNO_3 – soluble			
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–6	0.098	0.021	0.034	2.336	0.025	0.943	0.525	0.717	0.045	0.023	0.350	3.294
6–12	0.078	0.017	0.026	2.555	0.021	0.849	0.457	0.776	0.031	0.029	0.232	2.066
12–18	0.066	0.018	0.019	2.604	0.017	0.797	0.437	0.789	0.066	0.058	0.177	1.460
18–24	0.062	0.018	0.012	2.520	0.017	0.745	0.385	0.801	0.072	0.055	0.130	1.034

described, but this should be of no consequence for present purposes, since the larger lot was all taken from the same place and had been thoroughly mixed.

This particular soil was selected for the present investigation on account of its high content of organic matter and phosphorus, and the fact that a considerable supply of it was available.

Methods of analysis

Inorganic phosphorus. As the result of considerable experience with the method for inorganic phosphorus in humus solutions substantially as described by Potter and Benton, except that concentration of the neutralized nitric acid extract of the first magnesia mixture precipitate was omitted as unnecessary (it being easily possible with care to secure complete extraction and still not greatly exceed the volume of 125 cc. specified by the authors named), as well as the same method variously modified otherwise, the writer has adopted the following procedure:

Pipette a suitable aliquot (200 cc., representing 20 gm. of soil) of the humus extract into a 500-cc. centrifuge bottle, add 25 cc. of standard phosphate solution (approximately 0.0025 gm. P) and precipitate with 25 cc. of magnesia mixture, added drop by drop with stirring. Shake well and add strong ammonia to bring the concentration of NH_3 in the final volume of solution up to 2.5 per cent. Stopper and let stand three days. Whirl in the centrifuge at 3000 revolutions per minute for 10 or 15 minutes. Pour the supernatant liquid through an 11-cm. filter (Swedish Paper No. 0-B is satisfactory) carefully fitted into a 60° long-stemmed funnel with platinum cone in bell jar, and wash the inside of the bottle and surface of the precipitate once with cold water, being careful not to disturb the cake of precipitate. After pouring this through the paper, the latter may be washed several times with water and a little filter paper pulp added to the filter. Add to the precipitate in the bottle 25 or 30 cc. of dilute nitric acid (80 cc. of acid of specific gravity 1.2 diluted to 1000 cc.), shake well and allow to stand for a few minutes. Pour into the filter and receive the filtrate in a 250-cc. beaker covered with a perforated watch-glass under a bell jar; if the filtrate is not perfectly clear, it should be returned to the filter. Wash the bottle and precipitate with five or six more portions of the dilute nitric acid, using 15 or 20 cc. each time.

The filtration should not be unduly hastened at this time and it is better to use only enough suction to keep the stem of the funnel filled. The chances of loss will then be less, while the acid has an opportunity to diffuse into the precipitate and extract the soluble phosphorus with a minimum amount of washing. For the same reason, the funnel should be allowed to empty each time, but should then be refilled at once, otherwise the precipitate may become compact and crack, allowing channels to form.

The filtrate is neutralized with strong ammonia, and 8 cc. of nitric acid, specific gravity 1.2, is added; the volume should not exceed 175 cc. Add about 10 gm. of solid ammonium nitrate, heat to 60°C., add 50 cc. of warm neutral molybdate (75 gm. of crystallized ammonium molybdate per liter, dissolved with the aid of a little ammonia and neutralized with HNO_3) or 50 cc. of ordinary acid molybdate neutralized to litmus paper with ammonia, and maintain at 60° for about one-half hour. In case the latter is used, it is not desirable to add any solid ammonium nitrate. Remove from the bath and let stand several hours or over night. Filter, wash well with cold 1 per cent NH_4NO_3 slightly acidified with nitric acid, and dissolve back into the same beaker with 2.5 per cent NH_3, followed by washing with water, but keeping the volume as small as possible. Neutralize with HCl, add 5 cc. of 5 per cent citric acid solution to aid in keeping in solution any iron which may be present, make slightly alkaline with ammonia and precipitate according to the usual procedure with 10 cc. of magnesia mixture and one-tenth volume (about 10 cc.) of strong ammonia. After standing over night, the precipitated magnesium ammonium phosphate is filtered off, using a close-grained paper, as the precipitate may be in a very finely-divided condition, thoroughly washed with 2.5 per cent ammonia, redissolved with dilute hydrochloric acid into a clean beaker, keeping the volume of the solution below 100 cc. if possible. The phosphorus is reprecipitated by magnesia mixture as before, and after standing over night the white precipitate is filtered off on dense ashless paper (blue ribbon quality), thoroughly washed with 2.5 per cent NH_3 (one volume of strong ammonia, diluted to 10 volumes is sufficiently near the exact strength) and finally ignited and weighed as $Mg_2P_2O_7$. The weight of phosphorus is calculated from this, that corresponding to the phosphate solution added and the blank being deducted to obtain the net content of inorganic phosphorus of the 200-cc. aliquot taken. If desired, the determination might just as well be finished by any of the standard methods of weighing or titrating the yellow precipitate, beginning with the washed white precipitate following the neutral molybdate separation.

The writer's experience has been that it is not necessary to make any special effort to remove silica; the repeated precipitations and washings accomplish this perfectly, and none has ever been found in the final precipitate.

The above-described method differs very materially in several respects from that published by Potter and Benton; no ammonium chloride except that contained in the magnesia mixture and but one-half the quantity of the latter reagent prescribed by the authors named, is required. The object of this substitution is to avoid as far as possible, the "salting out" of the humus bodies and the consequent increase in difficulty of working with the larger precipitate. Reducing the proportion of magnesia mixture still further would reduce the amount of organic matter precipitated to an almost negligible quantity, but unfortunately cannot be done, as recovery of the inorganic phosphorus is then incomplete. The strength of ammonia employed and the time allowed are both much greater than advised in the original method; as will be shown subsequently, practically the same strength of ammonia and almost as much time are required to secure maximum extraction of the organic phosphorus compounds of the soil under investigation. In so far as stability is concerned, there has been no sensible increase in the content of inorganic phosphorus in a number of 4 per cent ammonium hydroxide extracts after standing several months.

The single washing of the first precipitate as directed in the procedure outlined is easily accomplished and seems to be all that is necessary; if thorough washing with water or dilute ammonia not containing a considerable amount of magnesia mixture is attempted, the precipitate begins to dissolve after the greater part of the salts present is removed, and it is possible with patience to wash practically all the organic matter out. The precipitated organic matter does not seem to have formed any definite compound with any of the reagents, but has merely been coagulated by the considerable concentration of salts.

The addition of a known amount of phosphate is for the purpose of having sufficient inorganic phosphorus present to enable that already present to precipitate. This would be unnecessary in case a soil giving an ammonia extract containing a considerable amount of inorganic phosphorus was being worked with.

In illustration of several of the points which have been mentioned, the following account of the distribution of the phosphorus in the several stages of the determination of inorganic phosphorus will be of interest.

In the determination 200-cc. aliquots of a humus solution, containing in that quantity 9.8 mgm. of total phosphorus, were treated as described, phosphorus being added to each, but 25 cc. and 10 cc., respectively, of magnesia mixture used; all were made to 2.5 per cent NH_3 and stood three days before centrifuging and filtering.

In table 2 account should be taken of the added phosphorus, which has not been deducted from figures representing the inorganic phosphorus found.

The results for inorganic phosphorus from the determinations in which 25 cc. of magnesia mixture were employed, besides being of a very different order of magnitude from those obtained by the use of but 10 cc. of the re-

agent, show a better agreement. In fact, the lack of agreement between the duplicates and the fact that in the case of two determinations in which the minimum quantity of magnesia mixture was employed, the amounts of inorganic phosphorus found are less than the amounts added, is evidence that the results of these determinations are of a lower degree of accuracy, although not absolute proof that the results are incorrect, since a poor method will often afford closely-agreeing results and in any method involving so many successive reprecipitations of a small quantity of the element determined, it may be expected that the tendency will be toward low results.

The figures obtained from determinations of total phosphorus in the filtrates from the first precipitation by magnesia mixture indicate that the larger amount of the reagent has precipitated about 60 per cent of the organic phosphorus along with the inorganic, while in the case of those determinations

TABLE 2

Distribution of phosphorus in the determination of inorganic phosphorus

MAGNESIA MIXTURE USED	INORGANIC PHOSPHORUS		"ORGANIC PHOSPHORUS"		
	Added	Found	Not precipitated	Remained in precipitate	Leached out
cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
25	2.5	3.9	3.7	4.5	0.2
	2.5	3.9	3.6	4.8	
	5.0	6.4			
	5.0	6.5			
10	2.5	2.7	8.8	0.6	0.2
	2.5	2.3	9.3	0.6	0.1
	5.0	4.4			

in which the precipitation was made by 10 cc. of magnesia mixture, only about 8 per cent of the apparent content of organic phosphorus was precipitated.

J. Stewart (21) appears to have been the first to apply the magnesia mixture precipitation procedure of Forbes and associates to the determination of inorganic phosphorus in alkali extracts of soil; he was led to abandon the method on account of the considerable solubility of the organic matter precipitated by magnesia mixture in the acid-alcohol prescribed by the Forbes method, and the large proportion of the total phosphorus precipitated by magnesia mixture. The present writer's experience with the acid-alcohol extraction of the Forbes method has been exactly that of Stewart; not only is the extract highly colored, but a voluminous precipitate of organic matter, iron, etc. appears when a second precipitation by magnesia mixture is attempted, while a gummy residue is left on evaporation. The second objection to the magnesia precipitation method would have more weight if it were shown that any considerable part of the organic phosphorus in the magnesia mixture

precipitate is extracted by the dilute acid employed for leaching out the inorganic phosphorus and carried on to the next stage; fortunately, the dilute nitric acid substituted by Potter and Benton for the acid alcohol of Forbes is not open to this objection, as shown by the data in table 2. Of the phosphorus precipitated by the larger amount of magnesia mixture, the greater part remains with the insoluble organic matter after leaching with acid, as shown by total phosphorus determinations following wet digestions of the leached precipitates. The figures for organic phosphorus taken into solution by the leaching with dilute acid were obtained by difference; apparently no more organic phosphorus was dissolved during the acid leaching when much was present in the precipitate leached than when but little was present.

A possibility that the larger amount of inorganic phosphorus found when 25 cc. of magnesia mixture was used is due to organic phosphorus taken into solution during the acid leaching and decomposed during subsequent operations, must be considered. As evidence again this, the following observations are presented.

1. In working with similar solutions of about the same organic phosphorus content, but containing less inorganic phosphorus and to which none had been added in the course of the determination of inorganic phosphorus, as large a precipitate of organic matter resulted as was encountered when much inorganic phosphorus was present. Nevertheless, even when magnesia mixture was used at the rate of 40 cc. per 200 cc of humus solution, the determination of inorganic phosphorus showed a mere trace or none at all. As the next step in the analysis, the neutral molybdate precipitation, has always been found capable of showing the presence of a very minute amount of inorganic phosphorus, it is evident that the decomposition of organic phosphorus compounds has been a negligible factor in these cases.

2. The ammonia extract, with which the results given in table 2 were obtained, was a mixture of extracts left from other work, in which known amounts of inorganic phosphorus were added to suspensions of acid-extracted soil in ammonia previous to filtration. While there is no means of knowing the proportion of inorganic phosphorus present in this mixture, except as the result of the analyses which have been discussed, all the data at hand indicate that it was greater in amount than the maximum shown when only 10 cc. of magnesia mixture was depended upon to precipitate it.

For a time, the writer made a practice of using the minimum quantity of magnesia mixture for this work, both for the reasons discussed by Stewart and on account of the ease with which the filtration and leaching by dilute acid are accomplished when the precipitated inorganic phosphorus is accompanied by little organic matter; the impossibility of obtaining consistent results, no matter how well the duplicate determinations agreed, was proof that something in the procedure was at fault.

The very small amount of organic phosphorus which accompanied the inorganic phosphorus in the nitric acid leachings of the first magnesia mixture

precipitate cannot have much influence upon the final results, even if all of it should appear as inorganic phosphorus. The ordinary acid molybdate separation is therefore found to be satisfactory in work with this soil. Fair results were also obtained when the molybdate separation was entirely omitted, a second precipitation by magnesia mixture in presence of citric acid to hold iron in solution being substituted.

This second precipitate of magnesium ammonium phosphate is but slightly contaminated by organic matter if care has been taken to insure a clear acid extract of the first, and was ignited and weighed directly.

If subsequent work should show that phytin or similar phosphorus compounds occur in alkali extracts of soil, the acid-alcohol separation could doubtless be successfully applied to this second precipitate.

Total phosphorus. Both the magnesium nitrate method of ignition and the wet combustion (Neumann) method have been used in obtaining the data for total phosphorus in alkali extracts of soil reported in this paper. The latter method is preferred, and was employed in the great majority of cases. It offers the advantages of total elimination of silica, no liability to loss by deflagration and, if properly conducted, none from other causes. The procedure followed is described:

Pipette a 200-cc. aliquot of the humus solution into a 500-cc. long-necked Kjeldahl flask, acidify with concentrated hydrochloric acid, add 50 cc. of concentrated nitric acid and 5 cc. of concentrated sulfuric acid. Drop in several bits of broken glass or beads as a preventive of bumping and place in the neck of the flask an "arrester" made by blowing a bulb of a size to fit loosely in the neck of the flask on the end of a piece of glass tubing and cutting the latter about six inches from the bulb; the tubing is bent at right angles about two inches from the end and one then has an arrangement which may be placed in the neck of the flask and held there by the short arm bent at a right angle when placed on the digestion rack with the mouth of the flask in the opening of the fume duct. The object is to prevent loss by spurting, to which these digestions are very liable, on account of the solid matter which separates during the later stages of the digestion.

The flask may be heated with a full flame from the beginning and the boiling proceeds without troublesome foaming or bumping. The solution should be allowed to boil until the sulfuric acid becomes concentrated, when the organic matter will be charred and copious fumes evolved. Turn out the flame and allow to cool for about 5 minutes; add a little concentrated nitric acid and heat as before, until the dense fumes have disappeared and only the colorless vapor of sulfuric acid is above the boiling acid. After several repetitions of this, always allowing the flask to cool somewhat before adding more nitric acid and never more than 2 or 3 cc. of this at a time, the contents of the flask should be practically colorless or only slightly tinged with yellow; allow to become quite cold, carefully dilute to 50 cc. with cold water, heat and boil gently for several minutes. Filter and wash into a 250-cc. beaker to a volume of about 125 cc. Neutralize with strong ammonia, bring any precipitate into solution again with nitric acid, add 15 gm. of solid ammonium nitrate, heat to 65°C. and precipitate with 50 cc. or more of official molybdate solution. Maintain at 65°C. for one-half hour, and allow to stand at least 3 hours longer before filtering. Filter, wash well with cold 1 per cent ammonium nitrate made slightly acid with nitric acid, dissolve from the filter with the aid of 2.5 per cent NH_3, add 5 cc. of 5 per cent citric acid solution and proceed in the customary manner for determination of phosphorus as magnesium pyrophosphate.

The small amount of sulfate present does not interfere with the complete precipitation of the phosphorus by the molybdate, provided the directions given are followed; the general effect of sulfates upon the molybdate precipitation is to cause the yellow precipitate to come down more slowly and to have an abnormal composition, necessitating more molybdate reagent and a higher temperature and longer time than would be required for the complete precipitation of phosphorus in the absence of sulfates. For this reason, the use of any of those methods for total phosphorus which depend upon the weighing or titration of the yellow precipitate itself, is not recommended unless the sulfates are first removed.

EXTRACTION OF ORGANIC PHOSPHORUS

In the search for a method for obtaining an index to the soil's total content of organically combined phosphorus, the use of alkalies as solvents for such phosphorus compounds suggests itself, since it is known that the alkali extracts of soils are often high in this element. In the past, much attention has been devoted to the phosphorus content of ammonia extracts of soil, and while no investigator has denied that at least a part of the phosphorus in such extracts of normal soils might be in organic combination, many explanations of its nature have been offered.

One of the most difficult features of the study of the ammonia-soluble constituents of the soil is getting rid of the clay, which remains in suspension in these extracts almost indefinitely; although it is readily enough precipitated by a sufficient addition of any one of a number of salts, there is no assurance that in precipitating it has not carried with it material from solution. On the other hand, this colloidal clay certainly contains in greater or less amount every constituent of the soil, and must be removed.

Gortner and Shaw (10) have recently laid considerable emphasis upon the relation of the clay content of alkali extracts of soil to the apparent occurrence of organic phosphorus as determined by Potter and Benton's method.

As an indication of the amount of study devoted to methods for the removal of this clay from solutions intended for humus determinations, it will suffice to say that a very considerable part of the voluminous literature upon humus has been devoted to this phase of the subject.

The following procedures for the removal of clay from ammonia extracts of soil have been studied in the present investigation:

1. Filtration through a layer of the soil itself supported by paper on a 25-cm. Büchner funnel, without any precipitant for the clay.

2. Centrifuging at 3000 revolutions per minute for 10 minutes in International Instrument Company's no. 3 laboratory centrifuge.

3. One passage through Sharples laboratory centrifuge running at 30,000 revolutions per minute.

4. Powdered ammonium carbonate added at rate of 2 gm. per liter, centrifuged like no. 2.

5. The same, 10 gm. per liter.

6. The same, 2 gm. per liter, filtered on a 25-cm. Büchner funnel.

7. The same, 10 gm. per liter.

8. Ammonium chloride added at the rate of 10 gm. per liter, centrifuged like no. 2.

9. Hydrogen sulfide passed into the suspension of soil in ammonia until clay was precipitated, centrifuged like no. 2.

10. The same, filtered on a 25-cm. Büchner funnel.

These humus extracts were prepared as follows:

One kilogram of soil was placed in a large bottle and digested with frequent shaking for several hours with 2 per cent HCl. It was then thrown upon a 25-cm. Büchner filter and washed with 1 per cent HCl until no calcium could be detected in a 50-cc. portion of the leachings. The soil was washed with water until the washings were entirely free from chlorine, sucked as dry as possible, and the cake of soil removed from the funnel and allowed to become air-dry, then reground. Then 85-gm. portions of this prepared soil were weighed into liter bottles, 850 cc. of 4 per cent NH_4OH added, and the mixture shaken during 4 working days in an end-over-end shaker revolving about 10 times per minute. The clay was thereafter separated as quickly as possible.

In table 3, the determinations made on these solutions are presented. The figures in the columns headed "grams" refer to the number of grams of the particular constituent per 200 cc. of humus solution. Humus and humus ash were determined by evaporating 200 cc. in a weighed platinum dish, drying at 110°C. for about $1\frac{1}{2}$ days, which was found to be the length of time required to approximate constant weight, weighing, igniting and again weighing.

Silica was determined by the standard method after a carbonate fusion of the ash. Alumina was determined by a modification of the Peters method, as described by Blair (2), and ferric oxide was determined by the colorimetric method described by Schreiner and Failyer (18) in a small aliquot of the filtrate from the silica. The humus solutions were compared as to color by diluting a suitable aliquot to 100 cc. and making the comparisons in a Schreiner colorimeter. Total and inorganic phosphorus were determined by the methods previously described.

The data for humus, presented in table 3, indicate that in those cases where centrifuging has effected a good removal of clay, the salts added to bring this about have caused a decrease in the content of organic matter in solution. The same effect is apparent in the figures for comparative color, and to an even greater extent in those for total phosphorus.

Centrifuging without the addition of any precipitant for clay, or only the minimum quantity of ammonium carbonate, gives high results for all constituents; in the case of humus, this is certainly partly due to combined water in the mineral matter. Considering the large amount of clay left in solution, the increase in the phosphorus content over a solution filtered without any precipitant is not large. Filtration after the addition of precipitants causes decreases in total organic matter, color and total phosphorus; the only ex-

ception to this is the solution treated with the minimum amount of ammonium carbonate, which has shown an increase in total organic matter. This effect of ammonium carbonate has been studied by MacIntire and Hardy (14), and is attributed by them to a solvent action upon a part of the organic matter of the soil which is not entirely dissolved by ammonia alone. With the larger amount of ammonium carbonate, this effect is masked by the tendency of high concentrations of the precipitant to cause occlusion of organic matter by the precipitated clay.

TABLE 3

Constituents of 200 cc. of 4 per cent NH_4OH *extract, representing 20 gm. of acid-extracted soil*

TREATMENT	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	RATIO OF SiO_2 TO Al_2O_3	TOTAL PHOSPHORUS
	gm.		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*
1. Filtered	0.7800	100	0.0632	0.0113	0.0025	0.0156	4.5	0.0090
2. Centrifuged	0.9070	125+	0.5718	0.2874	0.1350	0.0549	2.1	0.0095
3. Centrifuged	0.9185	109+	0.8599	0.4330	0.2055	0.0432	2.1	0.0092
4. 0.2 per cent $(NH_4)_2CO_3$ centrifuged	0.8686	114+	0.4382	0.2034	0.0960	0.0340	2.1	0.0093
5. 1.0 per cent $(NH_4)_2CO_3$ centrifuged	0.7755	95	0.0765	0.0215	0.0061	0.0119	3.5	0.0077
6. 0.2 per cent $(NH_4)_2CO_3$ filtered	0.7942	100	0.0647	0.0110	0.0027	0.0156	4.1	0.0087
7. 1.0 per cent $(NH_4)_2CO_3$ filtered	0.7488	89	0.0501	0.0098	0.0011	0.0109	8.9	0.0075
8. 1.0 per cent NH_4Cl centrifuged	0.7488§	91	0.0755	0.0231	0.0071	0.0119	3.3	0.0075
9. H_2S centrifuged	*	91	0.0903	0.0440	0.0031	0.0017	14.2	0.0080
10. H_2S filtered	*	94	0.0813	0.0369	0.0028	0.0015	13.2	0.0082
11. Original solution	0.8185	104	0.0777	0.0171	0.0032	0.0280	5.3	0.0092
12. Same filtered through bougie of porous porcelain	0.7311	85	0.0683	0.0137	0.0031	0.0176	4.4	0.0086
13. First liter to pass through Büchner filter	0.8281	100	0.0722	0.0175	0.0010	0.0233	17.5	0.0095
14. Second liter collected	0.8720	100	0.0777	0.0204	0.0010	0.0233	20.4	0.0096

+ Turbid from clay.

* Sulfur compounds present in large amount.

§ Ammonium chloride added has been deducted from weight.

The alumina content of these solutions is in all probability the best index to the actual amount of clay contained in them, although it is not at all impossible that a part of the alumina may be in solution in the ammonia instead of being merely in suspension as clay. The silica is in excess, indicating that in the greater number of cases it is not merely present as clay; in solutions 2, 3, 4, the clay content of which is highest, the ratio of silica to alumina is in each case 2.1, but in all other cases the ratio is much higher. The ferric oxide content of the solutions does not appear to bear any relation to other

constituents; it is highest where most clay is present, except in the case of solution 3, which is thought to have been diluted somewhat during the centrifuging. The results on this solution are anomalous in several respects and would not be included were it not that the composition of the ash is of interest, and to show the difficulty of removing all clay even by the application of great centrifugal force.

The reduction in the iron content of these solutions by precipitation with hydrogen sulfide is striking; it was noted that solution 10 was filtered through the layer of soil and paper on the Büchner funnel with unusual rapidity, which would seem to indicate that the iron content of these ammonia extracts contributes not a little to the difficulty of working with them.

Determinations of inorganic phosphorus in these solutions gave negative results; the work was done before the necessity of adding a known amount of phosphorus was appreciated. However, it is probable that the content of inorganic phosphorus is in every case quite small, and there is no reason to suppose that it differs to any extent among the solutions in the series. The content of organic phosphorus probably differs with the amounts of total phosphorus present, the phosphorus contained in suspended clay being included with the organic phosphorus.

The conclusion drawn from the data in table 3 is, that the procedure best adapted for obtaining an ammonia extract of the soil which contains the minimum amount of clay and the maximum amount of phosphorus includes as an essential feature separation of the clay by filtration through a layer of the soil itself supported by a flat paper filter on a Büchner funnel, substantially as described by MacIntire and Hardy.

The use of salts as precipitants for the clay before filtration causes decreases in the content of phosphorus without effecting any significant reduction of the clay content from that obtained by filtration without any precipitant. The use of the minimum quantity of ammonium carbonate (2 gm. per liter) has a slight influence upon both the organic matter and phosphorus content of the filtrate and in opposite directions. While the decrease in the amount of phosphorus present is but little greater than the probable difference in duplicate determinations, other solutions prepared similarly and compared show about the same difference; it may, therefore, be accepted as a fact that even this small amount of precipitant causes a noticeable decrease in the phosphorus content of the filtrate. Filtration is greatly facilitated when ammonium carbonate is used; 2 gm. per liter has almost as much influence in this respect as 10 gm. per liter.

A method of filtration which appeared promising and which it was hoped would remove every trace of clay depended upon the use of the Pasteur-Chamberland bougie of unglazed porcelain. This was not tried directly upon the soil suspension, but upon a solution already filtered on a Büchner funnel. The comparative analyses of this solution before and after filtration through the bougie are presented in table 3, nos. 11 and 12. The soil used was from a

lot prepared at a different time, so that the results are not strictly comparable with those for the other solutions tabulated.

Filtration through porous porcelain has reduced the amounts of all constituents present in the solution; the first portions to run through were apparently unchanged, but filtration gradually became slower and the filtrate lighter in color. About a liter of the solution was thus filtered; the last 50-cc. unfiltered portion remaining in the mantle was noticeably thicker and darker in color than the original solution had been.

On account of the slowness with which the filtrate passes through the layer of soil on the Büchner funnel when no precipitant is employed, indicating that a filter of this kind has but poor permeability, it was thought possible that there might be a similar decrease of concentration in the later portions to pass the filter. Nos. 13 and 14 in table 3 are, respectively, the first and second liters collected; it will be seen that the second liter is slightly stronger, instead of weaker than the first. A similar result, for humus and humus ash only, was reported by MacIntire and Hardy. These solutions were made from still another lot of extracted soil, which will explain the difference in composition observed when they are compared with solutions previously discussed and presented in table 3.

Optimum strength of ammonia

In table 4 data obtained from experimental work to establish the most favorable strength of ammonia are presented. The soil used was from a lot of 3 kgm. prepared at one time, in the manner previously described; it will hereafter be referred to as "lot 2."

The proportion of air-dry soil to ammonia solution was 1 gm. to 10 cc., as in the work previously discussed, and the shaking in the mechanical shaker continued during four working days. The solutions were filtered without precipitant on flat papers in 25-cm. Büchner funnels, these conditions having been tentatively adopted as standard.

The concentrations of ammonium hydroxide (NH_4OH) employed were, respectively, 0.5, 1, 2, 4, 6, and 8 per cent in numbers 1 to 6; there is a steady increase in humus and color extracted, with increases in the strength of ammonia; total phosphorus reaches a maximum at the 6 per cent strength; and the other ash constituents show a tendency to decrease with increases in the strength of ammonia.

Inorganic phosphorus determinations were made on all these solutions; both the absolute amounts and the differences observed were too small to have much significance, hence, the results are not tabulated. The results obtained did not indicate that the higher strengths of ammonia had caused any more decomposition of organic phosphorus thar the lower strengths.

The following conclusions are drawn:

Six per cent NH_4OH is but slightly, if any, more efficient than 4 per cent as a solvent for the organic phosphorus compounds in this soil; 6 per cent NH_4OH is as efficient as 8 per cent.

TABLE 4

Constituents of 200 cc. of ammonia extract, representing 20 gm. of acid-extracted soil

STRENGTH OF NH_4OH	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	RATIO OF SiO_2 TO Al_2O_3	TOTAL PHOSPHORUS
per cent	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*
0.5	0.6492	92	0.0928	0.0179	0.0057	0.0316	3.1	0.0078
1.0	0.6908	89	0.0788	0.0123	0.0010	0.0239	12.3	0.0080
2.0	0.7412	92	0.0760	0.0120	0.0018	0.0226	6.7	0.0083
4.0	0.8496	100	0.0664	0.0105	0.0012	0.0198	8.8	0.0089
6.0	0.8744	104	0.0680	0.0130	0.0014	0.0198	9.3	0.0090
8.0	0.8988	109	0.0648	0.0120	0.0015	0.0198	8.0	0.0090

Time soil should be shaken with ammonia

Data given in table 5 were designed to show the length of time necessary for maximum extraction of organic phosphorus.

Prepared soil from "lot 2" was employed, the standard conditions were followed, and the ammonia was 4 per cent ammonium hydroxide. The only variable factor was the length of time the soil and ammonia were shaken together in the end-over-end shaking machine.

The periods of shaking were 2, 4, 6, and 8 hours, and 2 and 4 days, respectively, for solutions 1 to 6.

Humus and color increase with the length of time shaken, the ash constituents tend to decrease, and the total phosphorus does not exhibit any significant variations.

The periods of time required for filtration varied with the time the soil and ammonia were shaken together; it is evident that the maximum deflocculation produced by 4 days' shaking has caused the layer of soil to form a

TABLE 5

Constituents of 200 cc. of 4 per cent NH_4OH *extract, representing 20 gm. of acid-extracted soil*

LENGTH OF TIME SHAKEN	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	RATIO OF SiO_2 TO Al_2O_3	TOTAL PHOSPHORUS
hours	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*
2	0.6972	88	0.0811	0.0195	0.0053	0.0368	3.7	0.0087
4	0.6957	90	0.0832	0.0223	0.0060	0.0257	3.7	0.0086
6	0.7074	91	0.0820	0.0224	0.0049	0.0263	4.6	0.0086
8	0.7715	94	0.0772	0.0208	0.0041	0.0235	5.1	0.0087
16	0.7945	98	0.0697	0.0153	0.0030	0.0227	5.1	0.0088
32	0.8173	100	0.0630	0.0133	0.0013	0.0209	10.2	0.0088

very impermeable filtering medium in the Büchner funnel, requiring a longer time for filtration and resulting in a more complete removal of clay.

In this connection, it should be noted that from one to three days' time was required for the filtrations, so that the soil and ammonia were actually in contact longer than stated. It may be, therefore, that 2 hours' shaking would not show such complete extraction of phosphorus if some quick method of separating clay had been employed.

Optimum ratio of soil to solvent

In order to determine what influence changes in the ratio of soil to solvent might have upon the extraction of the organic phosphorus, portions of prepared soil from "lot 2" were weighed out, representing 400, 100 and 20 gm. of moisture-free soil, and treated with ammonia solution of such volume and strength that there would be exactly 1 liter of 4 per cent NH_4OH in contact with the soil after allowing for the slight percentage of moisture in the soil and the reduction in strength of the ammonia by absorption of alkali by the acid-extracted soil. The mixtures were shaken for 8 hours and filtered in the customary manner, the contents of several bottles of the most dilute extract being filtered upon the same Büchner funnel.

TABLE 6

Constituents of 4 per cent NH_4OH *extract representing 20 gm. of moisture-free acid-extracted soil*

WEIGHT OF SOIL EXTRACTED BY ONE LITER	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	RATIO OF SiO_2 TO Al_2O_3	TOTAL PHOSPHORUS
gm.	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*
400	0.8309	433.0	0.0636	0.0093	0.0009	0.0238	10.3	0.0089
20	0.8734	17.5	0.1187	0.0456	0.0056	0.0268	8.1	0.0091
100	0.8185	100.0	0.0777	0.0171	0.0032	0.0280	5.3	0.0091

In table 6, data obtained from analyses of these solutions are presented; the figures in the columns headed "grams" refer to the number of grams of the particular constituent contained in a volume of solution representing 20 gm. of moisture-free soil. The figures for color represent a direct comparison of color of the extracts.

The 20-gm. per liter extract has removed most humus from the soil, but curiously enough, the 400-gm. per liter extract stands next. The latter has also extracted proportionately more color, and the 20 to 1000 solution has extracted proportionately least color. The ash, silica, alumina, and ferric oxide content of the 400 to 1000 solution is lowest per unit of soil; except for ferric oxide, the same constituents are highest in the case of the 20 to 1000 extract.

The extraction of phosphorus is a trifle less efficient when the volume of extract per unit quantity of soil is least; it has been the same, however, whether 20 or 100 gm. of soil were extracted by a liter of the solvent.

Preliminary extraction of bases

It is a well known fact that the solubility of the soil's organic matter in ammonia is increased if the soil has previously been leached with dilute acid. In order to determine whether or not the method of acid extraction has any influence upon the amount of organic phosphorus obtained in solution, the work recorded in table 7 was performed.

In the experiment, 100-gm. portions of the soil were placed in weighed bottles, and extracted as described:

1. Digested 4 hours with 1000 cc. of HCl to be tenth-normal at the end of digestion, washed free of chlorine with water on a 14-cm. Büchner filter.

TABLE 7

Data on phosphorus contained in 200-cc. aliquots of ammonia extracts, representing 20 gm. of soil

METHOD OF REMOVAL OF BASES	PHOSPHORUS		
	Total	Inorganic	Organic
	gm.	*gm.*	*gm.*
1. Digested with N/10 HCl, washed Cl free	0.0083	0.0012	0.0071
2. Digested with N/5 HCl, washed Cl free	0.0080	0.0007	0.0073
3. Digested with N/2 HCl, washed Cl free	0.0073	0.0005	0.0068
4. Digested and washed with 1 per cent HCl until practically all soluble removed, washed with water Cl-free	0.0079	0.0007	0.0072
5. Like (4), final washing with CO_2 solution	0.0079	0.0004	0.0075
6. Like (4), but washing with acid stopped with disappearance of Ca from leachings, washed with water	0.0080	0.0008	0.0072
7. Like (6), final washing with CO_2 solution	0.0080	0.0003	0.0077
8. Like (6) but washed twice only with 100-cc. portions CO_2 solution	0.0084	0.0010	0.0074
9. Digested like (1) washed twice only with water	0.0087	0.0016	0.0071
10. Digested like (2) washed like (9)	0.0085	0.0011	0.0074
11. Washed with formic acid, and water	0.0082	0.0016	0.0066
12. Washed with acetic acid, and water	0.0053	0.0017	0.0036

2. As before, HCl to be fifth-normal at the end of digestion.

3. As before, HCl to be half-normal at the end of digestion.

4. Digested with 1 per cent HCl, washed with the same until the leachings gave no precipitate with ammonia or ammonium oxalate, washed with water to the absence of Cl from washings.

5. Same as method 4, but final washing with saturated CO_2 solution, as suggested by Beam (1).

6. Same as method 4, except that acid washing stopped when calcium was no longer detected, final washing with water.

7. Like method 6, final washing with saturated CO_2 solution.

8. Like method 6 but washed twice only with saturated CO_2, 100 cc. each time.

9. Like method 1, washed with two portions of water only, 100 cc. each.

10. Like method 9, except that HCl was to be fifth-normal at the end of digestion.

11. Washed with formic acid equivalent in strength to 1 per cent HCl until leachings were free from calcium, acid washed out with water.

12. Like method 11, acetic acid instead of formic.

After sucking the cakes of soil on the Büchner filters as dry as possible, they were transferred back to the weighed bottles and the proper amount of strong ammonia and sufficient water added to make the weight that of a liter of 4 per cent NH_4OH, the weight of soil constituents removed by the previous acid extractions being allowed for.

The data recorded in table 7 include determinations of total and inorganic phosphorus, and figures for organic phosphorus by difference. It does not appear to make much difference how the extraction is performed, provided hydrochloric acid is used; in so far as any conclusions may be drawn from the slight differences observed, washing with 1 per cent acid until no calcium can be detected in 50 cc. of the leachings and washing the acid from the soil with saturated CO_2 solution has given the highest figure for organic phosphorus in both cases where this procedure was tested.

In two cases, digestion with N/10 HCl has resulted in a larger amount of total phosphorus but less organic phosphorus being taken into solution by the ammonia than has digestion by fifth-normal acid; in the single case where acid as strong as half-normal was in contact with the soil, the figures for phosphorus are lower, apparently indicating that acid as strong as this may cause solution or decomposition of organic phosphorus compounds.

A small amount of washing of the acid-extracted soil appears to be sufficient; the figures for organic phosphorus are the same, whether care was taken to wash out all chlorine, or whether but two portions of 100 cc. each were used for washing.

Formic acid appears to be less efficient than hydrochloric at equivalent strength; although total phosphorus in the ammonia extract is higher, the figure for organic phosphorus is lower.

Acetic acid is apparently very unsatisfactory.

Other methods for increasing the solubility of the organic matter in ammonia

The probable presence of a small amount of some organic phosphorus compound in the acid leachings of this soil, indicated both by determinations of total and inorganic phosphorus in acid extracts and by the fact that the acidified ammonia extracts of the soil contain much phosphorus in solution after separating the precipitated organic matter, which cannot be determined as inorganic phosphorus by the usual method, indicate that an efficient method for increasing the solubility of the soil's organic phosphorus in ammonia, avoiding the use of acid, would be desirable.

Fraps and Hamner (8) have described a method for increasing the solubility of humus by the addition of sodium phosphate to the alkali solution used for extraction; this method would not, of course, be suitable for the purpose of the present investigation, but it suggests the use of other precipitants for calcium as additions to the ammonia for this purpose. In table 8 data obtained from analyses of extracts made by the use of ammonium oxalate and of ammonium carbonate, each at the rate of 10 gm. per liter of 4 per cent ammonium hydroxide and 100 gm. of non-acid-extracted soil, are included.

Another procedure which suggested itself was the removal of the bases of the soil by digestion and washing with a solution of ammonium chloride, which would cause a part of the calcium and magnesium of the soil to be replaced by

TABLE 8

Constituents of 200 cc. of ammonia extract, representing 20 gm. of soil

TREATMENT OF SOIL	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	RATIO OF SiO_2 TO Al_2O_3	PHOSPHORUS		
								Total	Inorganic	Organic
	gm.		*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*
4 per cent NH_4OH with 1 per cent $(NH_4)_2C_2O_4.H_2O$	0.7210*	68	0.0316	0.0015	0.0032	0.0099	0.47	0.0024	0.0010	0.0014
4 per cent NH_4OH with 1 per cent $(NH_4)_2CO_3$	0.5428	50	0.0340	0.0015	0.0025	0.0068	0.60	0.0018	0.0010	0.0008
NH_4Cl-extracted	0.7808	93	0.0560	0.0048	0.0094	0.0358	0.51	0.0048	0.0014	0.0034
NH_4Cl-extracted	0.7832	96	0.0480	0.0051	0.0110	0.0134	0.46	0.0050	0.0016	0.0034
HCl-extracted	0.8432	100	0.0712	0.0076	0.0005	0.0596	15.2	0.0086	0.0006	0.0080
HCl-extracted	0.8220	100	0.0564	0.0090	0.0006	0.0162	15.0	0.0080	0.0004	0.0076
HCl in 80 per cent C_2H_5OH-extracted	0.6313	70	0.0524	0.0124	0.0029	0.0102	4.28	0.0083		
NH_4OH only	0.2700	12	0.0236	0.0032	0.0018	0.0006	1.78	0.0011		

* Determination of oxalate in small aliquots indicated that of 2 gm. of crystallized ammonium oxalate added to each 200 cc., 1.295 gm. remained; this has been deducted to obtain the above figure.

ammonium by interchange and thus removed. The ammonium chloride was afterwards removed by thorough washing with 80 per cent alcohol; neither the salt solution nor this diluted alcohol for washing extracted any color or appreciable amount of organic matter from the soil.

For comparison, there are included the analyses of 4 per cent NH_4OH extracts made from the same soil after the customary acid extraction, also one made after leaching the soil with 1 per cent hydrochloric acid in 80 per cent alcohol. Finally, to show the small amount of organic matter and phosphorus soluble in pure ammonia unless the soil is previously treated, data obtained from analyses of an extract made by digesting 100 gm. of soil with 1000 cc. of 4 per cent ammonium hydroxide, carbonate-free, are included.

By comparison of the data for the solution mentioned last with data obtained from analyses of solutions prepared in the same way except that additions of 10 gm. per liter of ammonium oxalate and carbonate were made to the ammonia used for extracting the soil, it is seen that the additions named have been quite effective in increasing the solubility of the soil constituents in ammonia although not to nearly so great an extent as the regular acid extraction. The oxalate has been more effective than the carbonate, as indeed might be expected from the slighter solubility of the corresponding calcium compound.

The ammonium chloride extraction has apparently been very effective in increasing the amounts of total organic matter and color taken into solution; it is not, however, proportionately effective in increasing the solubility of the organic phosphorus.

The ammonia extract of the sample which was previously extracted by 1 per cent hydrochloric acid in 80 per cent alcohol is quite low in total organic matter and color. The failure of the ammonia to extract more organic matter and color is attributable rather to previous removal of these constituents by the

TABLE 9

Calcium and magnesium extracted from soil by methods described

MANNER OF EXTRACTION	CALCIUM	MAGNESIUM
	per cent	*per cent*
Digestion and washing, 1 per cent HCl afterwards washed with water	0.6105	0.0704
Digestion and washing, normal NH_4Cl, afterwards washed with 80 per cent alcohol	0.5280	0.0482
Digestion and washing with 1 per cent HCl in 80 per cent alcohol, washed with 80 per cent alcohol	0.6080	0.0530

acid-alcohol than to poor removal of bases in the preliminary leachings. The amounts of calcium and magnesium extracted from 100 gm. of this soil by the several methods of extraction described are shown in table 9.

Similarly, it seems probable that the comparatively large amount of organic matter and color extracted from the soil previously leached with ammonium chloride solution is at least partly due to the fact that here practically no color and very little organic matter was removed from the soil during the preliminary leaching; even 1 per cent hydrochloric acid in aqueous solution removes more organic matter than would be desirable if the amount of "humus" in the ammonia solution were the only consideration.

The solutions which were prepared without the use of acid for preliminary extraction of bases are without exception low in silica, but contain more alumina, so that the ratio of silica to alumina is invariably less than 2.1, indicating that the alumina is in excess and that a part of it is present in a form other than clay, perhaps simply dissolved by the ammonia.

The amount of ferric oxide seems to follow to some extent other constituents soluble in ammonia; the very large amount present in one of each pair of solu-

tions prepared by acid and ammonium chloride extraction is an illustration of the variability of this constituent due to very slight differences in manipulation, these solutions not having been prepared at the same time as the others.

The content of inorganic phosphorus is highest in those solutions made without previous acid treatment; it is probable that leaching with hydrochloric acid reduced the content of inorganic phosphorus in the ammonia extracts by removing inorganic phosphorus capable of going into solution in ammonia. Some of the data in table 13 lend support to this view.

None of the substitutes for acid extraction can be considered successful for increasing the solubility of organic phosphorus in the case of this basic soil; the best is less than 50 per cent effective as compared with acid extraction.

Use of alkalies other than ammonia as solvents for organic phosphorus

As was recently demonstrated by Gortner (9), there may exist wide differences between the solvent effects of different alkalies, or different concentrations

TABLE 10

Constituents of 200 cc. of alkali extracts, representing 20 gm. of acid-extracted soil

ALKALI	PHOSPHORUS			COLOR
	Total	Inorganic	Organic	
	gm.	*gm.*	*gm.*	
Normal KOH	0.0094			65
N/4 KOH	0.0104	0.0014	0.0090	67
Normal NaOH	0.0106			79
N/4 NaOH	0.0107	0.0015	0.0092	86
Normal LiOH	0.0098			96
N/4 LiOH	0.0106	0.0016	0.0090	83
Normal Na_2CO_3	0.0082			65
Normal $HNaCO_3$ saturated with CO_2	0.0028			22
N/4 $HNaCO_3$ saturated with CO_2	0.0011			20
Normal NH_4OH	0.0092	0.0001	0.0091	100

of the same alkali, upon the organic matter of the soil. The alkaline solutions enumerated in table 10 were employed as solvents for the alkali-soluble phosphorus of this soil, 100-gm. portions of the acid-extracted and dried soil being shaken during one working day with 1 liter of the several solutions and filtered on 25-cm. Büchner funnels.

Aliquots of 200 cc. were employed for determinations of total and inorganic phosphorus, the methods previously described being used, with such modifications as were necessitated by the large amounts of fixed alkali present in some cases.

In the cases of all the fixed alkalies, the fourth-normal solutions contained more total phosphorus than the normal solutions, indicating that the organic phosphorus is less soluble in the more concentrated solutions; in the case of sodium hydroxide the difference is too small to be considered, and this case

may be an exception. Normal sodium carbonate has a moderate solvent action, but the bicarbonate solutions saturated with CO_2 were much less efficient.

At the fourth-normal concentration, the hydroxides of the alkali metals caused more total phosphorus to be taken into solution than did normal ammonium hydroxide, but determinations of inorganic phosphorus show that as solvents for organic phosphorus, they cannot be considered superior to ammonia.

Ammonia is also the best solvent for color, although this may be due in part to the fact that the ammonia solution contained by far the greatest amount of iron, the stronger alkali solutions containing very little.

The solutions of the fixed alkalies all dissolved large amounts of silica and alumina from the soil.

Among the mixtures of alkaline hydroxide and soil, striking differences were observed in behavior on standing; the suspensions containing potassium hydroxide coagulated and settled, and were easily filtered; those containing lithium hydroxide coagulated, but settled to only a slight extent and were filtered with difficulty. Sodium hydroxide occupies an intermediate position between these extremes, while the suspension of soil in ammonia neither coagulated nor settled, and next to the fourth-normal lithium hydroxide was the most difficult to filter.

Completeness of extraction by one treatment

Fraps (5) and, more recently, Russell and Prescott (17) have studied the solubility of the inorganic phosphorus of the soil in dilute acids; they are agreed that the quantity of phosphorus taken into solution at the first treatment is not the total amount capable of being dissolved from the soil by the particular dilute acid employed as a solvent, but is the difference between that quantity and the phosphorus again fixed or absorbed from the solution by the soil. As the disturbing effect of this absorption is undoubtedly one of the great difficulties encountered in studies of the probable availability of the soil's natural supply of phosphorus based upon solubility in dilute acids, it will be of interest to know whether or not any similar effect is operative to prevent the complete extraction of the organic phosphorus of the soil by a single treatment with ammonia.

The plan adopted for the determination of this point consisted of making successive extractions of the same portions of soil, weighing the bottles and filtration apparatus at appropriate times in order to get the necessary data to correct for volume of the preceding extract left in the cake of soil on the filter and for any change in volume due to evaporation.

A quantity of acid-extracted soil from "lot 2" was weighed into each of two liter bottles, and the proper amount of ammonia solution added to make exactly 1 liter of 4 per cent NH_4OH in contact with 100 gm. of moisture-free acid-extracted soil. The mixture was shaken 8 hours and filtered on a

25-cm. Büchner funnel without any precipitant, the funnel being covered with a well-fitting glass plate. The cake of soil remaining on the filter was replaced in the bottle, again made to the proper weight with 4 per cent NH_4OH, allowing for matter extracted, and the process repeated.

In all, four successive extracts of the duplicate portions of soil, designated A and B, were thus obtained; the amount of each preceding extract mixed with the next was found to be 8 per cent. The error by evaporation was less than 1 per cent, and is included in the above correction.

In table 11 the data obtained from this experiment are presented.

The results indicate that each successive extraction has removed a slight additional amount of every soil constituent determined, allowance being made for the 8 per cent of the previous extract mixed with those after the first. The data for organic phosphorus only will be discussed in detail; the

TABLE 11

Constituents of 200 cc. of 4 per cent NH_4OH *extract, representing 20 gm. of moisture-free acid-extracted soil*

EXTRACT AND SAMPLE	HUMUS	COLOR	ASH	SiO_2	Al_2O_3	Fe_2O_3	PHOSPHORUS		
							Total	Inorganic	Organic
	gm.		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
First A	0.7798	107	0.1017	0.0331	0.0038	0.0264	0.0091	0.0004	0.0087
First B	0.7922	107	0.1152	0.0420	0.0040	0.0257	0.0091	0.0003	0.0088
Second A	0.1612	12	0.0441	0.0209	0.0027	0.0045	0.0012	0.0001	0.0011
Second B	0.1501	11	0.0294	0.0131	0.0005	0.0031	0.0010	0.0002	0.0008
Third A	0.0642	3	0.0224	0.0100	0.0006	0.0013	0.0004	0.0002	0.0002
Third B	0.0836	4	0.0247	0.0096	0.0008	0.0026	0.0004	0.0002	0.0002
Fourth A	0.0508	2	0.0249	0.0106	0.0007	0.0009	0.0002	0.0001	0.0001
Fourth B	0.0476	2	0.0232	0.0112	0.0005	0.0010	0.0002	0.0002	

second extracts vary more in their composition than is desirable, that from sample A being considerably higher in ash constituents than that from sample B, indicating that the filtration in this case was probably less effective. If the data for the second extract of sample A are used as a basis for calculations, the second extraction has brought into solution approximately 0.4 mgm. of organic phosphorus; if the data for the second extract B are used, then only about 0.1 mgm. has been brought into solution. Analyses of the third and fourth extracts indicate that not more than 0.1 mgm. of organic phosphorus has been brought into solution by each extraction.

Of a total of 9.3 mgm. and 9.0 mgm., respectively, for samples A and B brought into solution by four extractions, 8.7 and 8.8 mgm., or 94 and 98 per cent were contained in the first extract, indicating that the organic phosphorus contained in the first ammonia extract approximates the total amount which may be brought into solution by ammonia.

Organic phosphorus in acid leachings

Potter and Benton (15) found that the acid leachings of the soils investigated were free from organic phosphorus. In view of the importance of the question, it was considered advisable to determine whether this was also the case with the soil under investigation by the writer; accordingly, 500-cc. aliquots of the 1 per cent HCl leachings and water washings of this soil combined were precipitated by 50 cc. of magnesia mixture, and one-tenth volume concentrated ammonia after the addition of 10 gm. of crystallized tartaric acid to hold iron and aluminum in solution. After standing 3 days, the precipitate was filtered off, thoroughly washed, redissolved in dilute HNO_3 and precipitated by acid molybdate, the determination being finished in the usual manner.

The percentage of recovery by this method varied from 95 to 97, somewhat less than a centigram of total phosphorus being present in the 500-cc. aliquots of the several acid solutions examined.

A synthetic solution was prepared, corresponding as closely as possible in iron, aluminum, calcium, magnesium and phosphorus content to one of the acid extracts under investigation; by the same method, 99 per cent of the phosphorus content was recovered. The absolute amounts of phosphorus not determined as inorganic in these solutions are so small, however, that the organic phosphorus in the acid extracts of this soil may be considered a negligible quantity.

Nature of the ammonia-soluble phosphorus of the soil

In the preceding discussions of the phosphorus contained in these alkali extracts of soil, the term "organic phosphorus" has repeatedly been used, when the phosphorus in solution not present as the orthophosphate ion or determinable by the usual method for inorganic phosphorus, was meant. In a few cases, where the humus ash was unusually high and had a composition indicating the probable presence of considerable clay, the term "organic phosphorus" has been qualified by the statement that probably a little inorganic phosphorus contained in clay was included.

Efforts to learn the identity of the organic phosphorus compounds in the ammonia extract of this soil have so far been without success; when the ammoniacal extract is acidified with acetic acid and a small amount of picric acid added to precipitate proteins, as described by Levene (13), a brownish black precipitate is obtained, which was found to contain 7.5 per cent of the total phosphorus in the original humus solution. To the clear deep reddish brown liquid somewhat more than its own volume of 95 per cent alcohol was added and the mixture allowed to stand; the dark-colored voluminous precipitate which separated contained 37.8 per cent of the total phosphorus originally present. Repeated solution of this precipitate in ammonia and reprecipitation by acetic acid and alcohol did not lighten its color appreciably; when boiled under a reflux condenser with 10 per cent sulfuric acid and the tests

for the decomposition products of nucleic acid applied as described by Schreiner and Lathrop (19), orthophosphoric acid was the only one which could be positively identified. At almost all stages of these separations, voluminous gummy precipitates appeared, highly colored in most cases.

The method for separating the organic phosphorus compounds from ammonia extracts described by Jegorev (12) proved entirely useless in the present case.

In the absence, then, of any direct proof that all the phosphorus in ammonia extracts not determined as inorganic phosphorus is actually in organic combination, it will be necessary to consider all the other possible explanations of its state of combination.

The fact that added phosphate is completely recoverable is proof that the phosphorus not determined by the method for inorganic phosphorus is not inorganic phosphorus prevented from precipitating by any occult influence attributable to other constituents of the solution.

It has likewise been shown that it is possible to obtain these ammonia extracts with a high content of organic phosphorus but practically free from clay; this is proof that phosphorus enclosed in mineral particles and so protected from attack by dilute acid is not concerned.

Fraps (7), in his discussions of the nature of the phosphorus in the ammonia extract of soil, has directed attention to the surprising solubilities of the phosphates of iron and aluminum in ammonia and their relatively difficult solubility in dilute acids. Following additions of these phosphates to ammonia, and to an ammoniacal soil extract, it was found that in each case a considerable amount of the phosphate had been taken into solution, as shown by increases in phosphorus and iron or aluminum content. Determinations of inorganic phosphorus were made; it was found that the phosphorus thus added was completely recoverable as inorganic phosphorus.

Gortner and Shaw (10) offer as an explanation for the presence of phosphorus in a form not determinable by the method for inorganic phosphorus, the theory that phosphoric acid is adsorbed by colloidal organic matter from acid leachings during the preliminary extraction of bases, and being held in the adsorbed state after the organic matter has been removed from the soil by ammonia is thus included with the organic phosphorus, or if not so held is possibly again adsorbed by organic matter as soon as the precipitated magnesium ammonium phosphate and organic matter obtained by a magnesia mixture precipitation of an ammoniacal soil extract is made acid in leaching out the inorganic phosphorus. As evidence opposed to this theory, attention is directed to the fact that added phosphate is recovered quantitatively as inorganic phosphorus irrespective of the actual amount added, within wide limits, and with a constant increment corresponding to the solution's original content of inorganic phosphorus. If adsorption is a factor of importance in the present connection, the figure representing the original content of inorganic phosphorus in the solution would not remain a constant with variations

in the amounts of phosphate since concentration of the substance adsorbed in the solution is one of the factors governing the amount of the substance removed from the solution by an adsorbent.

This statement is made with knowledge of the results reported by Prescott (16) for adsorption of phosphoric acid from N/20 HNO_3 by precipitated humus, in which the amount of P_2O_5 adsorbed shows no consistent relation to the concentration of this constituent and in fact is nearly a constant. Prescott neither considers the possibility of the presence of organic phosphorus in his humus preparation, nor the possibility of chemical precipitations by constituents of the humus solution; in either event, the amount of phosphorus precipitated on acidifying might be almost constant, regardless of the amount of P_2O_5 added.

Further evidence that the phosphorus of alkali extracts of soil not determinable as inorganic phosphorus is not adsorbed by humus or mineral colloids is found in the fact that upon acidifying the alkali extract, only a part of the phosphorus is precipitated, but determinations of inorganic phosphorus in the clear solution separated show even less inorganic phosphorus than was originally in the solution. As has been mentioned, acidification with acetic acid caused 7.5 per cent of the total phosphorus to be precipitated; hydrochloric acid precipitated a much larger amount, 44 per cent in one case. As acidifying with hydrochloric acid causes a larger precipitate of organic matter than is produced by the use of acetic acid, it was thought that repeated solution in ammonia and reprecipitation by hydrochloric acid might bring more phosphorus into solution; four repetitions of this treatment reduced the amount in the precipitate to 40 per cent of the total phosphorus present.

A 1 per cent sodium hydroxide extract of the same soil, the organic phosphorus content of which was about the same as that of the ammonia extracts described, about 9 mgm. per 200 cc., was acidified with hydrochloric acid; the phosphorus content of the precipitate was 14 per cent of the total amount originally in solution.

Determinations of inorganic phosphorus in these acid filtrates indicated no appreciable decomposition of the organic phosphorus compounds by the treatment.

The data discussed were obtained from work on humus solutions prepared in the customary way; in some cases, phosphorus was added to the finished solution, before the analyses were made. The results obtained from ammonia extracts prepared from soil to which phosphorus had been added before the alkaline extraction was begun should be of interest, because in this case the phosphorus is undoubtedly adsorbed in part under conditions similar to those postulated by Gortner and Shaw. Two cases present themselves:

a. Phosphorus is absorbed from neutral or alkaline solution; here the absorption may possibly include chemical precipitation as well as adsorption.

b. Phosphorus is adsorbed from acid solution and acid with excess of phosphorus removed by washing with water.

a. Both acid-extracted and unextracted soil were used for this experiment; two 100-gm. portions were placed in bottles, 1000 cc. of 4 per cent NH_4OH added to one bottle of each pair and 1000 cc. of 4 per cent NH_4OH containing ammonium phosphate equivalent to 10.5 mgm. of phosphorus added to the other bottles. The mixtures were shaken at intervals for several weeks, when 10 gm. of powdered ammonium carbonate was added to each bottle, the contents well shaken and transferred to centrifuge bottles. The centrifuged extracts were free from clay; data obtained from determinations of total and inorganic phosphorus are presented in table 12.

From the data in table 12, it appears that of the 2.1 mgm. of phosphorus added to each 200 cc. of solution, only 0.4 and 0.6 mgm. as shown by the determination of total and inorganic phosphorus, respectively, remain after contact with the unextracted soil. The unextracted soil is able to remove from the alkaline solution about 75 per cent of the added phosphorus in this case; the acid-extracted sample under similar circumstances apparently possesses no power of fixation, as the excess by both total and inorganic determinations

TABLE 12

Phosphorus in 200 cc. of 4 per cent NH_4OH *extract, representing 20 gm. of soil*

SAMPLE	TOTAL	INORGANIC	ORGANIC
	mgm.	*mgm.*	*mgm.*
Unextracted, check	1.8	0.2	1.6
Unextracted, phosphorus added	2.2	0.8	1.4
Acid-extracted, check	6.9	0.1	6.8
Acid-extracted, phosphorus added	9.0	2.2	6.8

corresponds to the amount added. The presence of inorganic phosphorus in the solution at the moment of extraction by alkali causes no increase in the figure for organic phosphorus.

b. The soil for this experiment had been acid-extracted and dried; two 100-gm. portions were shaken with 1000 cc. of N/5 HNO_3, and two portions with the same reagent containing, in each 200 cc., 5 mgm. of phosphorus in the form of ammonium phosphate. The mixtures were mechanically shaken for half a day, let stand over night, filtered on 14-cm. Büchner funnels and the cakes of soil washed with somewhat less than a liter of water, which was ample for the removal of soluble acid. The filtrate and washings were made to 2000-cc. and 400-cc. aliquots removed for determinations of total phosphorus, which aliquots correspond to 20 gm. of the sample.

The cakes of soil in the Büchner filters were transferred back into the bottles and sufficient ammonia and water added to make the volume 1000 cc. and the strength 4 per cent NH_4OH in contact with the 100 gm. of soil. These mixtures were mechanically shaken for the greater part of two days and finally filtered on 25-cm. Büchner funnels without the use of any coagulant. Corresponding portions from the same lot of acid-extracted soil, but not sub-

jected to the second extraction by N/5 HNO_3, were extracted by ammonia in the same way and at the same time.

The data obtained from this experiment are presented in table 13.

Fifth-normal nitric acid followed by water washing was able to extract 1.1 mgm. of phosphorus from each 20 gm. of the soil which had already been once extracted. The addition of 5.0 mgm. of phosphorus to the nitric acid did not raise the phosphorus content to 6.1 mgm. after contact with the soil and addition of washings; only 5.3 mgm. was found, indicating that 0.8 mgm. of phosphorus was held by the soil in such a way that neither digestion with dilute acid containing some phosphorus nor a reasonable amount of washing with water was able to remove it. If adsorption really is the factor responsible for the retention of phosphorus in cases such as this, as claimed by Russell and Prescott (17), then one is justified in the conclusion that this phosphorus is adsorbed. Ammonia extractions of the residues of soil following these second acid treatments afford data indicating that this adsorbed phosphorus is completely extracted by ammonia and such ammonia-soluble phosphorus is determinable as inorganic phosphorus.

TABLE 13

Phosphorus contained in extract representing 20 gm. of soil

METHOD OF EXTRACTION	TOTAL	INORGANIC	ORGANIC
	mgm.	*mgm.*	*mgm.*
(A) N/5 HNO_3	1.1		
(B) N/5 HNO_3 containing 5.0 mgm. P	5.3		
4 per cent NH_4OH extract of residue A	9.4	0.7	8.7
4 per cent NH_4OH extract of residue B	10.3	1.8	8.5
4 per cent NH_4OH	9.8	1.1	8.7

Composition of ammonia extracts of four depths of soil

Ammonia extracts of the four depths of the Paulding soil were prepared; 100-gm. samples were digested with 500 cc. of 1 per cent HCl for 4 hours with frequent shaking, filtered and washed on 13-cm. Büchner funnels with 1 per cent HCl until leachings were free from calcium, and finally washed with saturated CO_2 solution until chlorine-free. The cakes of soil were placed in bottles and water and strong ammonia added to make the strength 2.5 per cent NH_3 and the volume of solution in contact with the soil 1000 cc.; the mixtures were shaken 8 hours in a machine and finally filtered on 25-cm. Büchner funnels without the addition of any precipitant. The composition of the extracts is shown in table 14; no analyses of the humus ash were made. In table 15, the humus content, color and organic phosphorus content are presented in the form of ratios. The close correspondence of color, humus content and organic phosphorus are noteworthy and indicate that the ammonia-soluble organic matter of this soil has a very similar composition in all the depths sampled. Furthermore, the total nitrogen content of the four depths of the soil stands in similar ratio.

TABLE 14

Constituents of 200 cc. of 2.5 per cent NH_3 extract, representing 20 gm. of soil

DEPTH	HUMUS	ASH	PHOSPHORUS		
			Total	Inorganic	Organic
inches	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0–6	0.6588	0.0596	0.0077	0.0009	0.0068
6–12	0.4132	0.0648	0.0062	0.0010	0.0052
12–18	0.2920	0.0740	0.0052	0.0015	0.0037
18–24	0.2068	0.0636	0.0039	0.0015	0.0024

TABLE 15

Ratios of humus, color and organic phosphorus in ammonia extracts, and total nitrogen in soil

DEPTH	HUMUS	COLOR	ORGANIC PHOSPHORUS	NITROGEN IN SOIL
inches				
0–6	100	100	100	100
6–12	63	62	76	66
12–18	44	44	54	51
18–24	31	33	35	37

Organic phosphorus indicated by other data

Various investigators have considered the increase in the amount of phosphorus dissolved from a soil by acid extraction following ignition to represent organic phosphorus; while Fraps (6) has shown that this is not necessarily true, it will be of interest to note the effect of ignition upon the solubility of the phosphorus in the four depths of the soil under consideration. Twenty-gram portions of soil were heated in a muffle at about 500°C. for one-half hour; similar portions of unignited soil were used as checks; the method adopted for extracting the soluble phosphorus consisted of an overnight digestion with 200 cc. of cold 2 per cent HCl, followed by filtration on a 10-cm. Büchner funnel and washing with cold 1 per cent HCl to a total volume of 800 cc. Phosphorus was determined in the entire filtrate by the usual method after evaporating with nitric acid to expel chlorine.

The acid employed for the experiment described is very dilute in comparison with the 12 per cent acid employed for a similar purpose by Stewart (20); it was thought better to use the more dilute acid in order to avoid solution of organic phosphorus from the unignited samples. The procedure of washing with the acid following digestion instead of digestion alone, was intended to reduce to the minimum the amount of adsorbed phosphorus remaining in the extracted soil.

The data obtained from determinations of phosphorus in these extracts of the unignited and ignited samples are presented in table 16; it will be ob-

served that the quantities of phosphorus extracted from the unignited samples in the three lower depths are very similar, and that the same is true of all four depths after ignition. The differences observed do not indicate that results obtained from determinations of ignition-soluble phosphorus would throw any light upon the amounts of organic phosphorus in this soil.

By reference to table 1, it will be seen that the total potassium content of the four depths of this soil is quite uniform; the soil and subsoil may therefore be considered to be of practically the same mineral composition, and the method for calculating the content of organic phosphorus proposed by Hopkins and Pettit (11), may be applied. The results obtained are considerably at variance from the indications afforded by analyses of ammonia extracts of the corresponding samples from the lower depths, although the correspondence for the first depth is very close; by deducting from the total phosphorus content of the lowest depth the amount of phosphorus indicated to be organic by analyses of the ammonia extract, and using the amended figure for mineral phosphorus as the base, the results may be expected to be somewhat

TABLE 16

Phosphorus extracted from 20 gm. of soil, not ignited and ignited

DEPTH	NOT IGNITED	IGNITED	DIFFERENCE
inches	*mgm.*	*mgm.*	*mgm.*
0–6	7.7	9.4	1.7
6–12	6.3	9.3	3.0
12–18	6.2	9.2	3.0
18–24	6.0	8.7	2.7

better. This was done in obtaining the figures for organic phosphorus by the calculations given in table 17, last column.

The figures for organic phosphorus in the two intermediate depths now show satisfactory agreement with those obtained from analyses of the ammonia extract, but indications for the surface sample are too high. Several explanations for this are possible:

1. Extraction of organic phosphorus by ammonia has been incomplete; this seems unlikely in view of the apparently satisfactory extraction in the case of the lower depths.

2. The surface layer is not the same in mineral composition as the samples taken at lower depths; this may be a partial explanation, as the total potassium content of the surface layer shows the greatest departure from the mean.

3. The calculation method, in addition to the first incorrect assumption that the subsoil is free from organic phosphorus, involves another, namely, that organic phosphorus, once formed, does not again revert to the inorganic state. It is quite possible that as much phosphorus as is indicated by the calculation method, as corrected, has once been in the organic state, although no more remains organically combined than is obtained in ammonia solution.

4. Gortner and Shaw call attention to the fact that not all the phosphorus of plants is organic, since potassium dihydrogen phosphate is present in many vegetable saps. There would thus be a concentration of inorganic phosphorus in the surface layer of the soil through the action of the plant in addition to the accumulation of organic phosphorus. In this connection, it should be noted that such is apparently the case, as shown by the larger amount of acid-soluble phosphorus in the surface soil, although the factors enumerated under subjects 2 and 3 above may apply here also.

TABLE 17

Organic phosphorus in soil, by several methods

DEPTH	NH_4OH-SOLUBLE ORGANIC	IGNITION-SOLUBLE ORGANIC	CALCULATED	
			Hopkins-Pettit	Corrected
inches	*per cent*	*per cent*	*per cent*	*per cent*
0–6	0.034	0.009	0.036	0.048
6–12	0.026	0.015	0.016	0.028
12–18	0.019	0.015	0.004	0.016
18–24	0.012	0.014		(0.012)

SUMMARY

In this paper, analytical methods adapted to the determination of total and inorganic phosphorus in ammonia extracts of soils are described.

A satisfactory method for separating clay from ammoniacal soil extracts, having in view the maximum content of organic phosphorus, has been determined.

The proper procedures and conditions for the preliminary removal of bases from the soil and extraction by ammonia solution in the preparation of ammoniacal extracts intended for the study of the soil's content of organic phosphorus have been determined.

It is shown that as solvents for the organic phosphorus of the soil studied, solutions of the hydroxides of the fixed alkalies are not superior to ammonia. One extraction by ammonia, following the proper procedure, is shown to remove practically all the organic phosphorus from the soil that is capable of being taken into solution by ammonia.

No consistent relations between the contents of ammonia-soluble organic matter (humus), humus ash, silica, ferric oxide and alumina in these solutions could be observed. No constant relation between total organic matter and organic phosphorus was observed in ammonia extracts prepared in various ways, although there was a general tendency for these to vary together.

Evidence is presented that inorganic phosphorus adsorbed by colloids, organic or inorganic, is not included in the apparent content of organic phosphorus, as determined by the methods described.

The data obtained indicate that the organic phosphorus, as determined from analyses of properly-made ammonia extracts, approximates to the probable content of organic phosphorus in the four depths of this soil sampled.

Determinations of humus, color and organic phosphorus in ammonia extracts of four depths of the soil indicate that these ammonia soluble constituents are present in about the same relative proportions in the four depths examined. The total nitrogen contents of the four depths of soil stand in ratios very similar to those exhibited by the ammonia-soluble constituents named.

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CROSS-INOCULATION OF LEGUMES

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Received for publication November 4, 1918

INTRODUCTION

The work reported on in this short paper deals with the cross-inoculation of the various members of the common legumes. The extent to which various legumes will cross-inoculate is a very practical problem. For instance, if the same organisms will cause the formation of nodules on all the clovers but one culture containing but one strain of organism will be necessary to infect the seeds, when a mixture of the seeds of such legumes as crimson, red, and white clover is planted. If cross-inoculation did not take place, three separate and distinct organisms would be necessary to produce the same results.

As early as 1904, Hopkins (4) appreciated the fact that a very great similarity existed between the nodules of two different plants. At that time, he demonstrated the fact that the nodule production and growth of alfalfa was very much the same as that of sweet clover. He also showed that the bacteria taken from the roots of sweet clover caused the formation of nodules on the alfalfa plant as well as did the organisms taken from the roots of alfalfa.

Garman and Didlake (2) in 1914 reported the results of an extensive work in determining to what extent the organisms of the various legumes would cross-inoculate. They concluded that the organisms which cause the formation of nodules on the roots of various legumes may be grouped under 6 different species.

From the results of their work in which they tested the extent to which the legumes will cross-inoculate, Burrill and Hansen (1) show that the nodule organisms are divided into 11 distinct groups. These investigators include the 6 different species as given by Garman and Didlake (2) as 6 of their groups, and add 5 more.

From the practical standpoint, in the cross-inoculation of legumes, three very important questions present themselves. These are:

1. Will the organisms causing the production of the nodules on the particular plants always infect all the members of that group?

2. Is the nodule production and the size and vigor of the plant, the roots of which produced nodules caused by the cross-inoculation, as vigorous and strong, respectively, as that produced by the inoculant of the plant by the organism of its own kind (that which is isolated from that specific plant)?

3. Will the organisms cause nodule production by cross-inoculation after the culture has been in storage, without transfers having been made continuously at regular intervals, as for instance, if cultures were kept in storage on an agar medium, as under commercial methods? Hence, how long a time will these organisms (by cross-inoculation) retain their vigor of causing nodule production? The first two questions named above will be considered in the present paper.

METHODS AND MATERIALS

Culture media

The culture medium upon which the various *Bacillus radicicola* were isolated and grown, was that which was perfected by the senior author[1] for the Mulford Biological Laboratories. This proved very satisfactory for a luxuriant as well as a rapid growth for most of the various species of the legume organism.

Isolation of organisms

The method for isolating the organisms from the nodules of the various legume plants was similar to the one employed by Harrison and Barlow (3). This was as follows: By means of small forceps, several medium-sized nodules were taken from the roots of a young plant and placed in a sterile petri dish. The nodules were washed very carefully several times with sterile water. After this washing, they were allowed to remain in bichloride-hydrochloric acid solution (1) for from two to four minutes, after which they were soaked in sterile water for four minutes. The sterilizing solution was then washed off with two more washings of sterile nitrogen-free solution. By means of a sterile scalpel the nodules were crushed on a sterile (flamed) slide. A loopful of this cloudy suspension was transferred to a tube of agar that had been liquefied (temperature of 43°C.). Several transfers were made from this tube to other liquefied agar tubes, several dilutions thus being made. The contents of these tubes were poured into petri dishes. After a few days, when individual colonies had appeared on the plates, transfers from these were made to agar slants. After these cultures had grown on the agar slants, they were carefully examined in Smith tubes and under a microscope for contaminations. If free from contaminating organisms, they were ready for use. If contaminated, they were plated out again.

All of the legume organisms herein considered were isolated from the roots of the plants by the above method.

[1] Koch, Geo. P. Comparison of various culture media for *B. radicicola* (Not yet in print.)

Plant tests

For testing nodule production on plants, the agar test-tube method, employed by Garman and Didlake (2), was tried, and proved quite successful. By this method, however, conditions, being for the most part anaerobic, would be much more unnatural than the conditions under which these organisms under ordinary growth and development usually exist. Hence, a method whereby the conditions were as natural as possible, was worked out and employed. This method which proved very successful for our plant tests was as follows: Earthenware pots, of $3\frac{1}{2}$ and $4\frac{1}{2}$ inch inside diameter, were filled with fine sand, to which the following inorganic salts were added: 12.5 gm. calcium carbonate, 10.0 gm. calcium phosphate, 5.0 gm. potassium sulfate and 1.3 gm. magnesium sulfate per 25 kgm. After the sand in the pots was saturated with water, the pots were carefully wrapped in heavy paper and sterilized in the autoclave for 2 hours at 15 pounds pressure on 2 successive days.

The pots, having been sterilized, were planted with sterile seed. The method of sterilizing the seeds was as follows: They were placed in a sterile wide-mouthed bottle and then soaked in bichloride-hydrochloric acid solution for from 3 to 7 minutes, after which time the sterilizing solution was washed from the seed. The seeds were then allowed to soak in sterile water for the same length of time that they were in the sterilizing solution, and were then washed three times in sterile water. The seeds were next planted with sterile platinum tipped forceps, those of the larger legumes being planted in the $4\frac{1}{2}$-inch pots, while those of the smaller seeds were planted in the $3\frac{1}{2}$-inch pots. After planting, the pots were carefully placed on sterile glass plates in the greenhouse and covered with sterile bell jars.

The seeds were infected soon after planting. These were infected by carefully washing the organisms from the cultural growth on agar slants with 7 cc. of nitrogen-free solution. This suspension of organisms was then transferred to the pots by means of sterile pipettes. After the plants had grown from 3 to 4 weeks, they were taken from the pots, the sand washed from the roots, and the roots very carefully examined for nodulation. Each determination (treatment) was made in duplicate or triplicate (two or three pots). Several series of controls, (pots with seeds not inoculated), were always made. If correct technique was carried out and they were free from contaminations the roots of the control plants which were not infected, should have had no nodules.

Each experiment embodying the cross-inoculation of each group of organisms was carried out at least three times, and the results herein reported are the final average of the several experiments.

RESULTS

Alfalfa group

According to previous investigators, this group comprises the plants of *Medicago*, *Melilotus* and *Trigonella foenum-graecum*. Since alfalfa, sweet clover and burr clover are the most common and probably the only legumes of this group that are of any practical importance, these were the only ones considered in this work.

TABLE 1

The results of the cross-inoculation of B. radicicola of the alfalfa group

KIND OF PLANT	RESULTS OF THE INOCULATION			
	Not inoculated	Alfalfa	Sweet clover	Burr clover
Alfalfa	−*	3+	3+	3+
Sweet clover	−	3+	3+	2+
Burr clover	−	1+	1+	2+

* Throughout this work (−) indicates no nodule production, (1+) fair nodule production, (2+) good nodule production, (3+) very vigorous nodule production.

The results, as shown in table 1 above, demonstrate the fact that each organism isolated from the particular plant of this group of legumes caused the formation of nodules on the other members of the group, as it inoculated its original host. There is, however, a rather marked difference in the extent to which the organisms isolated from the various plants cause such nodulation. It is apparent that the alfalfa and sweet clover plants were strongly inoculated by the organisms of all these plants. The burr clover plants produced very few nodules when inoculated with the alfalfa and sweet clover organism. In fact, several plants had no nodules at all.

Clovers (genus Trifolium)

This group comprises all the clovers of the genus *Trifolium*, namely: mammoth red, alsike, crimson, red, white and zigzag clover. But 4 of the most commonly grown were studied in these experiments; these were crimson, alsike, red, and white. The results of the experiments are shown in the table below.

The results as presented in table 2 demonstrate conclusively, that the *radicicola* organism of these 4 clovers of the genus *Trifolium* cross-inoculated very well, and the nodule production, resulting fron the infection produced by the cross inoculation, was as vigorous on each plant as in cases where the infection was produced by its own organism. The white clover plants, for instance, when inoculated with organisms isolated from the red clover had as many and as large nodules, and the plants were as vigorous as when the inoculation was made with the organism isolated from the white clover plants.

TABLE 2

The results of the cross-inoculation of B. radicicola of the trifolium clovers

KIND OF PLANT	RESULTS OF THE INOCULATION				
	Not inoculated	Crimson	Alsike	Red	White
Crimson	−	2+	2+	2+	3+
Alsike	−	3+	3+	3+	3+
Red	−	3+	3+	3+	3+
White	−	3+	3+	3+	3+

Pea-vetch group

This group represents plants of genii Pisum, Vicia, Lathyrus and Lens. Of this group, members of each genus which represented the common plants under cultivation, were employed in this work. These were vetch, sweet pea, Canada field pea and garden pea.

TABLE 3

Showing the results of the cross-inoculation of B. radicicola of the pea-vetch group

KIND OF PLANT	RESULTS OF THE INOCULATION				
	Not inoculated	Vetch	Sweet pea	Canada field pea	Garden pea
Vetch	−	3+	2+	2+	3+
Sweet pea	−	3+	3+	3+	1+
Canada field pea	−	1+	2+	3+	2+
Garden pea	−	2+	1+	2+	3+

The results presented above further substantiate the claims of previous investigators that the organisms of these four legumes, representing three genii of plants, will successfully cross-inoculate.

It is shown, however, that while we find that the organisms of this group cross-inoculate, in several instances the extent of nodulation, namely, the size and numbers of the nodules, is considerably less on the plant inoculated with a culture other than one of its own kind. This seems to be more marked in the case of the garden pea and Canada field pea plants than in that of the other legumes of this group. From these experiments, we would conclude that under ordinary conditions with the pea-vetch group, we could not expect as vigorous nodulation by using cross inoculation as by employing the organism of each plant directly.

Cowpea group

This group, according to Burrill and Hansen (1), entails not less than 9 different types of legumes. Of these, there are but 4 or 5 of any great importance in agriculture. These are the cowpea, Japan clover, velvet bean,

peanut and partridge pea. We have considered the first three of the above named in the work herein reported. These represent the genii *Vigna*, *Lespedeza* and *Mucuna*.

TABLE 4

Showing the results of the cross-inoculation of B. radicicola of the cowpea group

KIND OF PLANT	RESULTS OF THE INOCULATION			
	Not inoculated	Cowpea	Japan clover	Velvet bean
Cowpea	−	3+	2+	3+
Japan clover	−	−(?)	1+	1+
Velvet bean	−	3+	3+	3+

Upon examining the above results we find that in all but one case inoculation was produced. The one in question, Japan clover plants inoculated with the cowpea organisms, failed to produce nodules each time the experiment was made. The tests were not as good as might have been possible if the Japan clover plants had not been so small. With this group of legumes, we again realize an irregularity in the extent of nodule production. It will be seen that the nodulation of the Japan clover plants in all cases was more or less poor.

DISCUSSION OF RESULTS

The results of the experiments with four of the principle groups of legumes corroborate the data of previous investigators, namely, that the organisms of plants of each group cause the formation of nodules on every other member of that group. With several groups there seems to be considerable variation in the extent to which the nodulation caused by cross-inoculation takes place. There is little doubt but that the organism isolated from the alfalfa plant and the one isolated from the roots of sweet clover are identical. Although the organism taken from the nodules of burr clover, belongs without doubt to the same group as the alfalfa-sweet clover organisms, nevertheless, the several different isolations of the alfalfa and sweet clover organisms always produced less vigorous nodulation on the roots of burr clover than the nodulation which they caused on their own original plants. This fact, taken into consideration with the differences in the nodulation in the pea-vetch and the cowpea group, brings to light the possibility that the organisms in cross-inoculation must adapt themselves to the conditions of their new host plant. This might be accomplished in a few generations of plants. Again one organism might be much less vigorous with regard to the manner in which it is able to attack the root of the legume, than is another organism of the same specific type. Hence, the results produced on plants of the same kind would be very different.

SUMMARY

From the results of the experiments here reported, we summarize as follows:

1. *Bacillus radicicola* isolated from the roots of alfalfa, sweet clover and burr clover, all cross-inoculate. The alfalfa and sweet clover organisms cause but scant infection on the roots of burr clover.

2. The organisms isolated from any one of the 4 clovers, crimson, alsike, red, and white, caused as vigorous a nodule formation by cross-inoculation as upon its original host.

3. The organisms of the garden pea, vetch, Canada field pea and sweet pea cross-inoculated. By the cross-inoculation, the nodulation produced on Canada field pea and garden pea was not as vigorous as that resulting when the organisms isolated from each of these plants, respectively, were used.

4. With one exception, namely the Japan clover plant inoculated with the cowpea organism, the organisms of the cowpea group cross-inoculated.

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STATEMENT OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC., REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

Of SOIL SCIENCE, published monthly at Baltimore, Maryland for October 1, 1918.

STATE OF NEW JERSEY } ss.
COUNTY OF MIDDLESEX }

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Jacob G. Lipman, who, having been duly sworn according to law, deposes and says that he is the Editor of SOIL SCIENCE and that the following is, to the best of his knowledge and belief a true statement of the ownership, management (and if a daily paper, the circulation), etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, printed on the reverse of this form, to wit:

1. That the names and addresses of the publisher, editor, managing editor, and business managers are:

Publisher, Williams and Wilkins Company, 2419 Greenmount Avenue, Baltimore, Maryland.
Editor, Jacob G. Lipman, College Farm, New Brunswick, New Jersey.
Managing Editor, None.
Business Managers, None.

2. That the owners are: Trustees of Rutgers College, New Brunswick, New Jersey.

3. That the known bondholders, mortgagees, and other security holders owning or holding 1 per cent or more of total amount of bonds, mortgages, or other securities are: None.

JACOB G. LIPMAN, *Editor*.

Sworn to and subscribed before me this 21st day of September, 1918.

IRVING E. QUACKENBOSS, *Notary Public*.

[SEAL.] My commission expires July 5, 1920.